COCAINE-RELATED ALTERATIONS IN THE DOPAMINE AND GLUTAMATE SYSTEM AND ASSOCIATED BEHAVIOURAL DEFICITS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Study 1

Study 2

ABSTRACT

Converging evidence from human neuroimaging and preclinical studies points to drug-induced neuroadaptations in the mesocorticolimbic dopamine system and corticostriatal glutamate circuitry as the underlying cause for the development and maintenance of cocaine addiction in combination with predisposing genetic, developmental, and environmental factors (Volkow and Li, 2005). Accordingly, drug-related disruption of prefrontal cortex function has been suggested to account for the loss of willed behaviours manifested as compulsive drug use, cognitive deficits, decision-making impairment, and craving in dependent cocaine users (Goldstein and Volkow, 2011).

Beyond the role of dopamine in mediating acutely rewarding effects of addictive drugs and reward-related learning, dopamine has further been implicated in several cognitive functions and colour discrimination (Berridge and Robinson, 1998; Kelley, 2004). Prior studies demonstrated specific blue-yellow colour vision impairment (CVI) in dependent cocaine users and it was postulated that cocaine-induced changes in retinal dopamine might be responsible (Desai et al., 1997; Roy et al., 1996, 2003). However, it has not been examined if CVI even occurs in non-dependent cocaine users, whether it is specific for dopaminergic stimulants, and if it is related to cognitive performance. We aimed to clarify these issues in the first study (section 3) by measuring colour vision discrimination with the Lanthony Desaturated Panel D-15 Test and memory performance with the Auditory Verbal Learning Test in 47 recreational and 29 dependent cocaine users, 23 MDMA users, and 47 stimulant-naïve controls. Results revealed that both recreational and dependent cocaine users showed more frequent and more pronounced CVI, predominantly in the blue-yellow spectrum, compared to psychostimulant-naïve controls. Interestingly, only MDMA users with high exposure to dopaminergic stimulants exhibited CVI comparable to cocaine users, whereas pure MDMA users were largely unaffected. Verbal declarative memory performance was worse in cocaine users with colour vision disorder compared to users and controls with intact colour vision, while both CVI and cognitive deficits were found to be related to cocaine use. Overall, these results support the notion that even recreational cocaine and amphetamine use might induce blue-yellow CVI, whereas the serotonergic stimulant MDMA does not seem to impair colour vision. Moreover, the association between cognitive deficits and CVI may reflect a potential relationship between retinal and cerebral dopaminergic alterations.

Accumulating evidence from preclinical research indicates that enduring adaptations in the corticostriatal glutamate circuitry may be responsible for the persisting vulnerability to craving and relapse in cocaine users (Kalivas, 2009). In particular, the metabotropic glutamate receptor type 5 (mGluR5) has directly been linked to drug reinstatement in animal models and has gained interest as a potential target for new pharmacotherapies aimed at preventing relapse in human cocaine users as pharmacological antagonism of mGluR5s attenuated self-administration of cocaine in rats (Duncan and Lawrence, 2012; Kalivas and Volkow, 2011; Olive et al., 2012). To what extent preclinical findings are alienable to human cocaine users has not yet been investigated due to methodological constraints. Thus, in the second study (section 4), we investigated if human cocaine users exhibit altered density of the mGluR5 compared to

drug-naïve controls. To quantify mGluR5 availability in addiction-related brain areas, 18 male controls (12 smokers, 6 non-smokers) and 18 male participants who met the DSM-IV criteria either for cocaine abuse or dependence (13 smokers, 5 non-smokers) underwent positron emission tomography with the mGluR5-selective radioligand ^{11}C-ABP688 (Ametamey et al., 2006, 2007). Results indicated that cocaine use was not associated with altered mGluR5 density. In contrast, smokers exhibited marked global decreases with an average of 20% in mGluR5 density compared to non-smokers irrespective of cocaine use. The time interval since the last nicotine use was positively related to mGluR5 density in all brain regions of interest, reflecting that the decrease of mGluR5 availability was particularly pronounced in individuals who had smoked very recently. These results might have implications regarding the development of novel pharmacotherapies aimed at facilitating smoking cessation and indicate that more research is required to clarify if pharmacological antagonism may also have a beneficial effect in human cocaine users.

Behavioural studies have concordantly shown that dependent cocaine users exhibit decision-making deficits in the Iowa Gambling Task (IGT) and prefer immediate smaller rewards over larger delayed rewards (Bechara et al., 2002; Kirby and Petry, 2004; Verdejo-Garcia et al., 2007). If decision-making is compromised to a similar extent in non-dependent cocaine users and if these deficits extend to social decision-making was addressed in the third study (section 5). For this purpose, 68 recreational cocaine users, 30 dependent cocaine users, and 68 stimulant-naïve controls completed two classical, non-social decision-making tasks (IGT, Delay Discounting paradigm) and two social interaction paradigms adapted from economic tasks where money could either be distributed fairly or selfishly between oneself and an interaction partner (Distribution Game, Dictator Game). Decisions in the social interaction tasks of both cocaine user groups were more self-serving compared to controls. In contrast, only dependent cocaine users chose fewer advantageous cards in the IGT and preferred immediate smaller rewards over larger delayed rewards compared to controls. Cocaine consumption correlated negatively with non-social but not with social decision-making. Interestingly, particularly cocaine users with high craving scores acted in a more selfish manner in the money distribution games than controls. These results imply that social interaction deficits are already present in recreational users, while non-social decision-making deficits occur predominantly in dependent cocaine users. Moreover, correlations with drug consumption indicate that deficits in non-social decision-making could be partially induced by cocaine, whereas predisposing personality traits may mainly account for the more self-serving choices during social interaction in cocaine users.

Overall, these results imply that even non-dependent cocaine users may exhibit dopaminergic alterations, that in contrast, cocaine users do not manifest postsynaptic glutamatergic changes as measured by mGluR5 availability, and that on the behavioural level predominantly dependent cocaine users show impaired non-social decision-making, while both recreational and dependent cocaine users show more self-serving social decision-making. Altogether, these results have important implications for the development of novel pharmacotherapies aimed at preventing relapse in cocaine and nicotine users, and the conceptualization of prevention and treatment strategies, possibly precluding the transition to addiction in recreational cocaine users and ameliorating quality of life in dependent cocaine users.

ZUSAMMENFASSUNG

Konvergierende bildgebende Befunde aus dem Humanbereich und präklinische Studien weisen darauf hin, dass Substanz-induzierte Neuroadaptationen im mesocorticolimbischen Dopaminsystem und corticostriatalen Glutamat-Schaltkreis die Entstehung und Aufrechterhaltung der Kokainsucht, zusammen mit prädisponierenden genetischen, entwicklungsbedingten und Umweltfaktoren, fördern (Volkow and Li, 2005). Dementsprechend wurde postuliert, dass Funktionsbeeinträchtigungen des präfrontalen Cortex dem Verlust willentlichen Verhaltens in abhängigen Kokainkonsumenten unterliegen. Dies äussert sich als zwanghafter Drogenkonsum, kognitiven Defiziten, beeinträchtigtem Entscheidungsverhalten und intensivem Verlangen nach Kokain (Goldstein and Volkow, 2011).

Dopamin ist nicht nur zentral für die Verarbeitung von Belohnungseffekten süchtig machender Substanzen und belohnungs-assoziiertem Lernen, sondern ist auch in verschiedenen kognitiven Funktionen und der Farbdiskriminierung involviert (Berridge and Robinson, 1998; Kelley, 2004). Frühere Studien fanden spezifische Blau-Gelb-Farbsehbeeinträchtigungen (FSB) in abhängigen Kokainkonsumenten und es wurde angenommen, dass Kokain-induzierte Veränderungen der retinalen Dopamintransmission verantwortlich sein könnten (Desai et al., 1997; Roy et al., 1996, 2003). Bislang existieren jedoch keine Befunde, ob FSB auch in nicht-abhängigen Kokainkonsumenten auftreten, ob FSB spezifisch für dopaminerge Stimulanzien sind und ob sie mit kognitiver Performanz zusammenhängen. Wir untersuchten diese Fragestellungen in der ersten Studie (Abschnitt 3) indem wir in 47 gelegentlichen und 29 abhängigen Kokainkonsumenten, 23 MDMA-Konsumenten und 47 Stimulanzien-unerfahrenen Kontrollen die Farbsehdiskriminierungs-Fähigkeit mit dem Lanthony Desaturated Panel D-15 Test und Gedächtnisleistung mit dem Verbalen Lern- und Merkfähigkeitstest erhoben. Die Resultate zeigten, dass sowohl gelegentliche als auch abhängige Kokainkonsumenten verglichen mit der Kontrollgruppe häufigere und stärker ausgeprägte FSB vor allem im Blau-Gelb-Spektrum zeigten. Interessanterweise wiesen nur diejenigen MDMA-Konsumenten, welche zusätzlich dopaminerge Stimulanzien konsumiert hatten FSB auf, die vergleichbar mit den FSB der Kokainkonsumenten waren, wohingegen sich pure MDMA-Konsumenten als weitgehend unbeeinträchtigt zeigten. Die verbal-deklarative Gedächtnisperformanz war schlechter in Kokainkonsumenten mit Farbsehstörungen im Vergleich zu Konsumenten und Kontrollen mit intaktem Farbsehen und sowohl FSB als auch kognitive Defizite waren mit dem Kokainkonsum assoziiert. Insgesamt bestätigen diese Resultate die Annahme, dass bereits gelegentlicher Kokain- und Amphetaminkonsum eventuell Blau-Gelb-FSB induzieren könnten, wohingegen die serotonerge Substanz MDMA die Farbsehdiskriminierung nicht zu beeinträchtigen scheint. Darüber hinaus könnte der Zusammenhang zwischen kognitiven Defiziten und FSB eine potentielle Beziehung zwischen retinalen und zerebralen dopaminergen Veränderungen reflektieren.

Eine wachsende Anzahl präklinischer Befunde deutet darauf hin, dass andauernde Adaptationen des glutamatergen corticostriatalen Schaltkreises in Kokainkonsumenten für die persistierende Vulnerabilität gesteigerten Verlangens nach Kokain und für die häufigen Rückfälle verantwortlich sein könnten (Kalivas, 2009). Insbesondere der metabotrope Glutamatrezeptor Typ 5 (mGluR5) wurde direkt mit erneutem

Drogenkonsum in Tiermodellen assoziiert und gilt als potentieller Ansatzpunkt für auf Rückfallprävention abzielende Pharmakotherapien in der Kokainabhängigkeit, da gezeigt wurde, dass mGluR5-Antagonisten Kokain-Selbstverabreichung in Ratten verminderte (Duncan and Lawrence, 2012; Kalivas and Volkow, 2011; Olive et al., 2012). Inwiefern präklinische Befunde auf menschliche Kokainkonsumenten übertragbar sind, ist bisher nicht erforscht worden. Deshalb untersuchten wir in der zweiten Studie (Abschnitt 4), ob Kokainkonsumenten im Vergleich zu Drogen-unerfahrenen Kontrollprobanden veränderte mGluR5-Dichten aufweisen. Um die mGluR5-Verfügbarkeit zu quantifizieren, wurden 18 männliche Kontrollen (12 Raucher, 6 Nichtraucher) und 18 männliche Kokainkonsumenten die entweder die DSM-IV-Kriterien für Kokainabusus oder –abhängigkeit erfüllten (13 Raucher, 5 Nichtraucher) mit der Positronen-Emissions-Tomographie untersucht, bei welcher der mGluR5-selektive Radioligand ^{11}C-ABP688 (Ametamey et al., 2006, 2007) angewendet wurde. Die Resultate ergaben, dass der Konsum von Kokain nicht mit veränderter mGluR5-Dichte assoziiert war. Andererseits zeigten Raucher eine starke, globale Abnahme der mGluR5-Dichte von durchschnittlich 20% im Vergleich zu Nichtrauchern, unabhängig vom Kokainkonsum. Der Zeitraum seit dem letzten Nikotinkonsum war positiv mit der mGluR5-Dichte in allen untersuchten Hirnregionen assoziiert – die Abnahme der mGluR5-Verfügbarkeit war also besonders stark ausgeprägt in Teilnehmern, welche vor kurzem geraucht hatten. Diese Befunde könnten wichtige Implikationen für die Entwicklung neuer Pharmakotherapien haben, welche darauf abzielen die Nikotinabstinenz zu erleichtern. Darüber hinaus legen diese Resultate aber auch nahe, dass weitere Studien benötigt werden, um zu untersuchen, ob mGluR5-Antagonisten auch bei menschlichen Kokainkonsumenten wirksam sein könnten.

Behaviorale Studien haben konsistent gezeigt, dass abhängige Kokainkonsumenten in der Iowa Gambling Task (IGT) ein defizitäres Entscheidungsverhalten zeigen und dass sie sofort verfügbare, kleinere Belohnungen grösseren, später verfügbaren Belohnungen vorziehen (Bechara et al., 2002; Kirby and Petry, 2004; Verdejo-Garcia et al., 2007). Ob das Entscheidungsverhalten bereits in nicht-abhängigen Kokainkonsumenten beeinträchtigt ist und ob diese Defizite auch bei sozialem Entscheidungsverhalten auftreten, wurde in der dritten Studie (Abschnitt 5) untersucht. Zu diesem Zweck nahmen 68 gelegentliche und 30 abhängige Kokainkonsumenten, ebenso wie 68 Stimulanzien-unerfahrene Kontrollen an zwei klassischen, nicht-sozialen Entscheidungsaufgaben (IGT, “Delay Discounting” Paradigma) und an zwei sozialen Interaktionsparadigmen (Distribution Game, Dictator Game) teil. Die Interaktionsparadigmen wurden von ökonomischen Entscheidungsaufgaben adaptiert und es ging darum Geld entweder auf faire oder egoistische Weise zwischen sich und einem Interaktionspartner zu verteilen. Sowohl gelegentliche als auch abhängige Kokainkonsumenten trafen Entscheidungen in den sozialen Interaktionsaufgaben, die verglichen mit dem Entscheidungsverhalten der Kontrollen, eigennütziger waren. Im Gegenteil dazu wählten nur abhängige Kokainkonsumenten signifikant weniger vorteilhafte Karten im IGT und präferierten sofort verfügbare, kleinere Belohnungen gegenüber grösseren, später verfügbaren Belohnungen im Vergleich zu den Kontrollen. Kokainkonsummuster korrelierten negativ mit nicht-sozialem Entscheidungsverhalten, nicht jedoch mit sozialem Entscheidungsverhalten. Interessanterweise verhielten sich aber vor allem die Kokainkonsumenten mit gesteigertem, akutem Verlangen nach Kokain

(„craving“) egoistischer in den Geldverteilungsaufgaben verglichen mit den Kontrollen. Diese Resultate legen nahe, dass defizitäres soziales Interaktionsverhalten bereits in gelegentlichen Kokainkonsumenten vorhanden ist, wohingegen erst die abhängigen Kokainkonsumenten Beeinträchtigungen in nicht-sozialem Entscheidungsverhalten zeigen. Des weiteren weisen die Korrelationen mit den Drogenkonsumparametern darauf hin, dass die Defizite in nicht-sozialem Entscheidungsverhalten teilweise durch den Kokainkonsum induziert sein könnten, wohingegen möglicherweise vor allem prädisponierende Persönlichkeitseigenschaften für das eigennützigere Entscheidungsverhalten der Kokainkonsumenten in sozialen Interaktionen eine Rolle spielen.

Zusammenfassend implizieren diese Resultate, dass bereits nicht-abhängige Kokainkonsumenten möglicherweise dopaminerge Veränderungen aufweisen könnten, wohingegen Kokainkonsumenten verglichen mit den Kontrollen keine postsynaptischen, glutamatergen Veränderungen im Bezug auf die mGluR5-Verfügbarkeit aufweisen. Zudem weisen nur abhängige Kokainkonsumenten Beeinträchtigungen in nicht-sozialen Entscheidungsaufgaben auf, während bereits gelegentliche Kokainkonsumenten ein eigennützigeres Entscheidungsverhalten zeigen. Insgesamt könnten diese Resultate einerseits wichtige Implikationen für die Entwicklung neuer Pharmakotherapien haben, welche darauf abzielen Rückfälle bei Kokain- und Nikotinkonsumenten vorzubeugen. Andererseits sind sie aber auch bedeutsam für die Konzeptualisierung von Präventions- und Behandlungsangeboten, welche möglicherweise den Übergang von gelegentlichem zu abhängigem Konsum verhindern und die Lebensqualität in bereits abhängigen Konsumenten verbessern könnten.

ABBREVIATIONS

^{11}C-ABP688	3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-enone-O-carbon-11-methyl-oxime
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor
ACC	anterior cingulate cortex
DD	Delay Discounting
DLPFC	dorsolateral prefrontal cortex
fMRI	functional magnetic resonance imaging
iGluR	ionotropic glutamate receptor
IGT	Iowa Gambling Task
LD-15	Lanthony Desaturated Panel D-15
LTD	long-term depression
LTP	long-term potentiation
MDMA	3,4-methylenedioxy-N-methylamphetamine
mGluR	metabotropic glutamate receptor
mGluR5	metabotropic glutamate receptor type 5
MRS	magnetic resonance spectroscopy
NAcc	nucleus accumbens
NMDA	N-methyl-D-aspartate receptor
OFC	orbitofrontal cortex
PET	positron emission tomography
PFC	prefrontal cortex
VTA	ventral tegmental area
xc^{-}	cystine-glutamate exchanger

1

THEORETICAL BACKGROUND

1.1 Thesis outline

The present doctoral thesis was part of the Swiss National Foundation project "Neurosocial consequences of cocaine use: a longitudinal investigation" also referred to as Zurich Cocaine Cognition Study ($ZuCo^2St$) and was carried out under supervision of Prof. Dr. rer. nat. Boris Quednow, head of the Department of Experimental and Clinical Pharmacopsychology at the University Hospital of Psychiatry in Zurich. Behavioural experiments were conducted in the laboratories at the University Hospital of Psychiatry in Zurich and the positron emission tomography (PET) study took place at the Division of Nuclear Medicine under supervision of Prof. Dr. med. Alfred Buck at the University Hospital Zurich. The scope of the present thesis only captures selective aspects of the ongoing longitudinal study. Accordingly, data from the longitudinal study as well as the magnetic resonance spectroscopy (MRS) analyses will not be part of this thesis and will be published later.

The present thesis addresses the three broad research areas of dopaminergic, glutamatergic, and behavioural alterations associated with cocaine use, and is structured in six main sections: In the first section, the theoretical background is provided reviewing pertinent epidemiological findings, the definition of cocaine addiction, and the prevailing addiction model. Moreover, dopaminergic and glutamatergic neuroadaptations that are crucially involved in mediating the transition from controlled cocaine use to addiction hallmarked by compulsive drug use are delineated. Finally, relevant literature regarding decision-making deficits in cocaine users is summarized. Section 2 contains an overview of the original research conducted in the present thesis and outlines the aims and hypotheses of all three studies. The first study, delineated in section 3, addressed the relationship between blue-yellow colour vision discrimination and cognitive performance in recreational and dependent cocaine users, MDMA (3,4-methylenedioxy-N-methylamphetamine) users, and controls. In the second study, presented in section 4, we investigated if the density of the metabotropic glutamate receptor type 5 (mGluR5) differs in cocaine users and stimulant-naïve controls. And in the third study, presented in section 5, we examined the performance of recreational and dependent cocaine users in non-social and social decision-making tasks in comparison to stimulant-naïve controls. In the final section 6, the results of the three studies are summarized, methodological limitations and important implications for prevention and treatment strategies are discussed, and finally implications and suggestions for future research are proposed.

1.2 Pharmacology, pharmacokinetics, and acute effects of cocaine

Cocaine (benzoylmethylecgonine, $C_{17}H_{21}NO_4$), a potent psychostimulant drug, is a naturally occurring alkaloid extracted from the *Erythroxylon coca* plant, which grows in the Andes in South America (Egred and Davis, 2005; Restrepo et al., 2009). There are two main forms of cocaine resulting from different manufacturing processes. The water-soluble *hydrochloride salt* can also be used as local anesthetic and can be administered orally, intranasally, or intravenously. The second form of cocaine, the *freebase*, originates after cocaine is processed with ammonia or sodium bicarbonate (baking soda) and is also known as "crack" because it produces a cracking sound when being smoked (Carrera et al., 2004; Egred and Davis, 2005).

Depending on the route of administration, cocaine pharmacokinetics varies considerably. Extremely rapid absorption occurs after inhalation and intravenous injection, with 3 to 60 seconds until onset of action, a peak effect after 1 to 5 minutes, and an action-duration of 5 to 60 minutes, resulting in the highest addiction liability. Intranasal or mucosal administration has a much slower onset of action ranging from 1 to 5 minutes, a later peak effect of 15 to 20 minutes, and a slightly longer duration of action of 60 to 90 minutes (Egred and Davis, 2005). The half-life of cocaine is approximately 90 minutes as cocaine is rapidly metabolized by the liver, producing the metabolites benzoylecgonine and ecgonine methyl ester that account for 75-90% of the cocaine metabolism, as well as ecgonine, and the biologically active metabolite norcocaine (Carrera et al., 2004).

Cocaine blocks reuptake of monoamines by binding to dopamine, norepinephrine, and serotonin transporters, thereby increasing transmitter availability in the synaptic cleft (Harris and Baldessarinig, 1973; Ritz et al., 1990). The reinforcing effects of cocaine are thought to be mainly due to its action on dopamine transporters, resulting in subjectively experienced effects of euphoria, heightened energy, hypermotility, and inflated self-esteem, which are often followed by anhedonia, insomnia, irritability, and in some cases paranoia and psychotic symptoms (Carrera et al., 2004). Moreover, cocaine use can lead to a number of physical complications including cardiovascular, gastrointestinal, pulmonary, genitourinary and obstetric, neurological (infarction, seizures, migraine, haemorrhage), and musculoskeletal side effects, mainly due to vasoconstriction-mediated effects on noradrenergic neurotransmission (Egred and Davis, 2005; Knuepfer, 2003; Restrepo et al., 2009).

1.3 Epidemiology of cocaine use

Cocaine is the second most used illicit drug worldwide with an estimated 13 to 20 million users, corresponding to annual global cocaine use levels of 0.3-0.4 per cent (%) (UNODC, 2012). In Europe, it is estimated that 15.5 million people aged 15-64 years (4.6%) have used cocaine at least once in their life, 4 million (1.2%) people have used cocaine in the last year, and 1.5 million (0.5%) people have used cocaine in the past month. Interestingly, national figures for lifetime (0.3-10.2%) and last year prevalence rates (0.1-2.7) vary substantially, with most central and eastern European countries reporting low levels, whereas particularly Ireland, Spain, Italy, and the United Kingdom feature higher prevalence rates than the European average (EMCDDA, 2012). For Switzerland, the Federal Ministry of Health reported cocaine use lifetime prevalence rates of 3% (4% for men, 2% for women), a last year prevalence of 0.4%, (0.7% men, 0.2% women), and a last month prevalence of 0.2% in people aged 15-64 years (BAG, 2012). Not surprisingly, cocaine use among young European adults aged 15-34 years is slightly higher than in the age group of 15-64 year old people, with an estimated lifetime prevalence of 6.3%, a last year prevalence of 2.1%, and 0.8% for last month use. It is noteworthy, that cocaine use in young adults is particularly high among young males, amounting to a male to female ratio of two to one (EMCDDA, 2012). In Switzerland, cocaine is also used most frequently among young adults aged 15-34 years. The last year prevalence reported for young Swiss adults aged 15-19 years was 0.7%, 1.3% for adults aged 20-24 years, and 0.9% for adults aged 25-34 (BAG, 2012). International comparisons reveal that the average last year European prevalence rate of cocaine use (2.1%) is below reported figures from Australia (4.8%) and the United States (4.0%), but comparable to Canada (1.8%) (EMCDDA, 2012). Increasing trends in last year prevalence rates of cocaine use over the past decade were observed in European countries reporting the highest cocaine use, but also the United States and Australia. Peak levels were around 2008/2009 and prevalence rates appear to be declining to some extent in most countries, with the exception of Australia, and will have to be monitored closely (EMCDDA, 2012).

As evidenced by the presented epidemiological data, there are substantial numbers of non-addicted recreational cocaine users as well. Although not everyone will undergo the transition to dependence, cocaine is classified as a highly addictive drug (Nutt et al., 2007), and it is estimated that around 21% of cocaine users will meet dependence criteria by the age of 45 years (Wagner and Anthony, 2002). The high prevalence rates of cocaine use are also reflected by the high treatment demand. Accordingly, 17% of all patients, who entered drug treatment programs in the USA (SAMHSA, 2011), and 23% of the drug-seeking patients in Europe, did so for the treatment of stimulant addiction (EMCDDA, 2011).

1.4 Definition of cocaine addiction

Cocaine addiction and addiction in general is a chronically relapsing disorder that is characterized by compulsive seeking and taking of the drug despite the encounter of adverse consequences, the inability to control drug intake, and emergence of a motivational withdrawal syndrome when access to the drug is prevented that is associated with a negative emotional state (e.g., anhedonia, irritability, anxiety, craving) (APA, 2000; Koob and Volkow, 2010).

Although different types of addictive drugs may be associated with unique neuroadaptations, they share many common aspects, wherefore a general addiction model will be reviewed in this section and molecular alterations, specifically in association with cocaine use, will be the subject of the following two sections. Converging evidence from animal and human studies have revealed that drug use is associated with a myriad of neuroadaptations in brain circuits implicated in reward and motivation, memory, conditioning, habituation learning, executive function and inhibitory control, interoception and self-awareness, as well as stress reactivity and that these alterations may ultimately result in drug addiction also referred to as drug dependence (Koob and Volkow, 2010). It is noteworthy that only 10-20% of individuals who use drugs will escalate their drug use patterns and become addicted (Volkow et al., 2005). The transition to addiction as well as the course and severity of addiction is thus largely dependent on interacting effects of genetic, developmental, and environmental factors, as well as the type of drug (Koob and Volkow, 2010; Volkow et al., 2005).

1.5 Prevailing addiction model

As early as 2002, Goldstein and Volkow proposed the *impaired response inhibition and salience attribution* (iRISA) syndrome as an explanatory framework for addiction. According to this model, drug-induced disruption of prefrontal circuits that are crucially involved in regulating subcortically located limbic reward regions and hold a pivotal role in higher-order executive functions, results in loss of self-directed/willed behaviours manifested as compulsive drug use, decreased sensitivity to non-drug related, natural reinforcers, while excessive salience is attributed to the drug itself as well as drug-related cues (Goldstein and Volkow, 2002, 2011).

Based on the preclinical and human neuroimaging findings emerging over the past ten years, Koob and Volkow (2010) elaborated on the iRISA model by providing more detailed information regarding which specific brain circuits are involved in the different addiction stages (Fig. 1). They proposed that the drug addiction cycle is composed of three stages that shall be briefly outlined (Koob and Volkow, 2010).

Binge/intoxication stage: Most drugs with abuse liability are initially used because of their hedonic properties or alternatively due to the desire of group conformity (Müller and Schumann, 2011; Koob and Volkow, 2010). The reinforcing effects of addictive drugs are mainly mediated by the mesolimbic dopamine projection from the ventral tegmental area (VTA) to the ventral striatum [embodying the nucleus accumbens (NAcc)] (Di Chiara and Imperato, 1988). More specifically, drugs have the ability to potently increase extracellular dopamine in the NAcc by blocking the dopamine transporters. In concordance, human brain imaging studies have revealed that drug-induced increases in dopamine in the striatum, are associated with the subjective experience of euphoria, pleasure, and being high (Volkow and Wang, 1996).

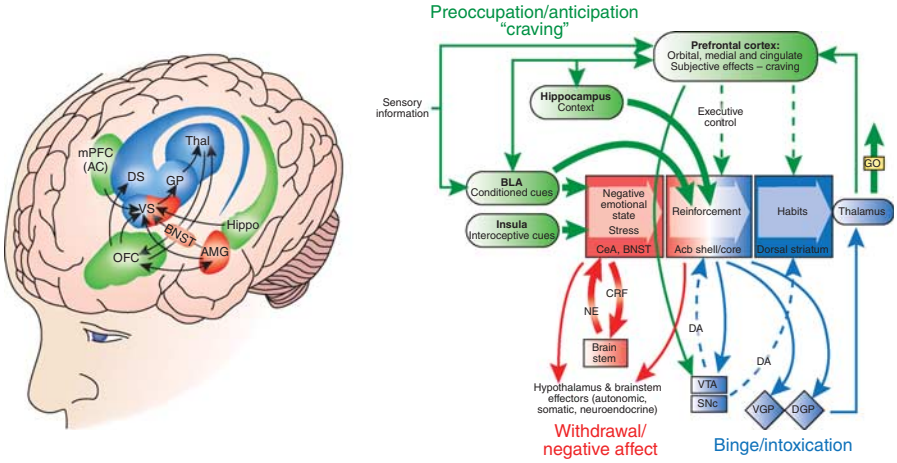

Fig. 1. Neural circuitry associated with the **binge/intoxication stage, withdrawal/negative affect stage,** and the **preoccupation/anticipation (craving) stage** of the addiction cycle. Green/blue arrows, glutamatergic projections; red arrows, dopaminergic projections; Acb, nucleus accumbens; BLA, basolateral amygdala; VTA, ventral tegmental area; SNc, substantia nigra pars compacta; VGP, ventral globus pallidus; DGP, dorsal globus pallidus; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; NE, norepinephrine; CRF, corticotropin-releasing factor. Reprinted by permission from Macmillan Publishers Ltd: Koob and Volkow, 2010, Neuropsychopharmacology Reviews 35(4): 217-238, copyright 2010.

Particularly drugs with fast pharmacokinetic properties leading to rapid dopamine increases are associated with the described subjective responses and are more likely to be addictive (Volkow et al., 1995). Therefore, rapidly absorbing drugs with a short half-life, as is the case with cocaine, are often repeatedly administered in a binge-like pattern due to their relatively short-lasting effects. Furthermore, a very important feature of the dopamine system is the ability to establish associations between neutral stimuli and the drug. Accordingly, drug-associated cues can elicit a comparable dopamine reaction as the drug itself, which may persist even after tolerance arises and drug effects are weakened (Volkow et al., 2011; Wong et al., 2006). Findings from animal studies revealed that

the amygdala and hippocampus are important anatomical substrates for the establishment of drug-cue associations (Koob, 2009a; Koob and Volkow, 2010). Another key mechanism that develops after repeated drug use is the shift from ventral to the dorsal circuits in the striatum, a structure that mediates stimulus-response habits (Yin and Knowlton, 2006; Yin et al., 2009). It is important to note that emerging preclinical findings have provided evidence that in contrast to the rewarding effects of drugs that are primarily dependent on the dopamine system, drug-seeking behaviour, which could be regarded as an equivalent to human relapse is mainly mediated by glutamatergic projections from the PFC to the NAcc (for review see Kalivas, 2009), which will be discussed in detail in section 1.7. In summary, the binge/intoxication stage may partly be initiated by impulsive notions individuals may exhibit resulting in *positive reinforcement* learning due to the rewarding effects inherent to drugs of abuse (Koob, 2009).

Withdrawal/negative affect stage: After drug intoxication and particularly after bingeing behaviour, drug users experience a motivational withdrawal syndrome characterized by dysphoria, emotional distress, irritability, and sleep disturbances. Notably, the extent of withdrawal symptoms differs substantially depending on frequency and chronicity of drug use, as well as the type of drug (Koob and Volkow, 2010). The extended amygdala, which is composed of the bed nucleus of the stria terminalis, the central nucleus of the amygdala, and possibly a transition zone in the medial portion of the NAcc, appears to induce stress and anxiety-like effects via its neurotransmitters and neuropeptides including corticotropin-releasing factor, norepinephrine, and dynorphin and projects to the hypothalamus and brain stem (Koob and Volkow, 2010). Studies with addicted humans addressing mechanisms underlying *acute withdrawal* have reported enhanced sensitivity to the effects of GABA-enhancing compounds possibly indicating down-regulation of GABA transmitters (Volkow et al., 1998) as well as decreases in endogenous opioids during cocaine withdrawal that may potentially contribute to negative affect (Zubieta et al., 1996). Moreover, during *protracted withdrawal* imaging studies have consistently demonstrated hypofunction in the dopamine system encompassing decreased D_2 receptor availability in the striatum (up to 4 months after the last use of cocaine) and decreased dopamine release, which may account for the blunted sensitivity to natural reinforcers and the observed amotivation in chronic drug users (Martinez et al., 2004; Martinez et al., 2005; Volkow et al., 1997; Volkow et al., 2007). Protracted detoxification has also been associated with disrupted activity in frontal brain regions, which is hypothesized to undermine willed inhibitory control and adaptive behavioural responses. Interestingly, lower levels of D_2 receptors were found to be associated with decreased metabolism in the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) in chronic cocaine users (Volkow et al., 1990, 1993; Volkow et al., 1997) and relative cerebral blood flow was significantly lower in the prefrontal cortex (PFC) and the left lateral frontal cortex in cocaine users compared to controls (Volkow et al., 1988). In sum, the withdrawal/negative

affect stage can be regarded as *negative reinforcement* learning, prompting addicted individuals to seek drugs anew in order to avoid negative emotional states.

Preoccupation/anticipation (craving) stage: Addicted individuals manifest high vulnerability to relapse even after prolonged abstinence and chronic relapse is one of the major difficulties in addiction therapy (Langleben et al., 2008). Conditioned drug-associated cues or stress have the ability to trigger the preoccupation/anticipation stage and may induce strong drug craving, which in turn makes relapse more likely. Several brain circuits are said to be involved and may interact. Preclinical studies suggest that drug-induced reinstatement depends on the glutamatergic circuit involving the projection from the PFC to the NAcc core (Baker et al., 2003; Kalivas, 2009), while cue-induced reinstatement is mediated by the basolateral amygdala, which in turn projects to the PFC (Everitt and Wolf, 2002; Weiss et al., 2001), and stress-induced drug reinstatement in animals has been associated with activation of corticotropin-releasing factors and norepinephrine in the extended amygdala (Shaham et al., 2003; Shalev, Grimm, and Shaham, 2002). The preoccupation/anticipation (craving) stage has primarily been associated with disrupted PFC function in humans. Indeed, a myriad of imaging studies in humans have highlighted the role of the PFC in exerting executive function by guiding and controlling adaptive behaviour. It has been hypothesized that the *dorsal PFC* [including the dorsal ACC, dorsolateral prefrontal cortex (DLPFC), and inferior frontal gyrus] is particularly important with regard to top-down control and meta-cognitive functions, the *ventromedial PFC* (including the subgenual ACC and medio-orbitofrontal cortex) is predominantly involved in emotion regulation such as assigning incentive salience to drugs and drug-associated cues, while the *ventrolateral PFC and lateral OFC* mediate impulsivity and automatic response tendencies (Goldstein and Volkow, 2011). Although craving is not sufficient for relapse and difficult to measure in absence of drug-paired cues (Koob and Volkow, 2010), functional magnetic resonance imaging (fMRI) studies in humans have revealed that the orbital and anterior cingulate cortices, and the temporal lobes including the amygdalae are activated during acute craving (Childress et al., 1999; Garavan et al., 2000; Grant et al., 1996; Maas et al., 1998; Wexler et al., 2001; Wilson et al., 2004). Despite accumulating preclinical knowledge that the glutamate circuitry may hold a pivotal role in mediating craving and relapse behaviour, due to the lack of suitable glutamatergic markers, merely nothing is known about potential glutamatergic neuroadaptations in humans. To date, the only study investigating glutamatergic alterations in crack cocaine users by means of using MRS has reported lower free glutamate levels in the ACC in comparison to controls (Yang et al., 2009). In order to expand the knowledge of how the glutamate system may become altered in cocaine users, in the second empirical study of this thesis we conducted a PET study where we quantified the availability of the mGluR5, which is discussed in detail in section 1.7.4.1 and 4.

In summary, the addiction cycle may be initiated and dominated by *impulsivity* at early stages, and may gradually shift to *compulsivity* at later stages due to incisive neurodaptations. Because of the rewarding effects inherent to drugs of abuse, early stages are accompanied by *positive reinforcement* driving the motivation to obtain drugs, while later stage are associated with *negative reinforcement* where automatic behaviours are manifested to alleviate the negative affective state occurring during withdrawal (Koob, 2009).

In the next two sections, more detailed information about underlying molecular adaptations in the dopamine and glutamate system that may mediate the transition from occasional, controlled drug use to compulsive, addicted drug use will be provided. These two sections provide important background knowledge for the first two empirical studies of the present thesis.

1.6 The mesocorticolimbic dopamine system in cocaine addiction

1.6.1 The role of dopamine in reward

The role of dopamine in reward processing has been extensively researched and is well established. The *mesolimbic dopamine pathway* from the VTA to the NAcc is the most important projection for cocaine-related reward. In addition, also the *mesostriatal pathyway* projecting from the substantia nigra into the dorsal striatum, and the *mesocortical pathway* where dopamine cells located in the ventral tegmental area project to the frontal cortex, further contribute to the rewarding effects of drugs of abuse (Wise, 2009). Several ^{11}C-raclopride PET studies have shown that higher dopamine increases, indirectly measured via occupancy of D_2 receptors in the striatum, were associated with more pronounced subjectively experienced high or euphoria. As mentioned in sections 1.2 and 1.5, reinforcing effects of cocaine also depend on pharmacokinetics. The faster cocaine reaches the brain, the more intense the subjective high (Volkow et al., 2009).

1.6.2 The role of dopamine in normal learning and conditioning of drug-associated cues

It has become clear that the role of dopamine goes beyond the mediation of hedonic pleasure and that the immediately rewarding effects of cocaine are not sufficient to induce addiction. In fact, dopamine signalling is crucially involved in assigning an event with salience, in facilitating learning by mediating the acquisition of memories, and the development of appropriate behavioural responses that either result in approach to or retreat from an important stimulus. Notably, natural reinforcers also have the ability to increase dopamine in the NAcc. However, there are two main differences between dopamine release following the occurrence of a natural stimulus and following exposure to

cocaine. First, dopamine release by cocaine is characterized by a much greater amplitude and longer duration beyond normal physiological limits that can be elicited by a natural stimulus (Kalivas and O'Brien, 2008). Second, cocaine always leads to an increase in dopamine (with the exception of periods after cocaine binges leading to depletion of dopamine), while tolerance develops to the release of dopamine by biological stimuli (Kalivas and O'Brien, 2008; Martinez et al., 2007). Once a person has learned how to obtain a natural reward, further dopamine release to facilitate learning becomes obsolete (Deutch and Roth, 1990; Schultz, 2004). Interestingly, although no dopamine response may be elicited upon obtaining the natural reward, dopamine can still signal the arrival of a reward by conditioned stimuli. For instance, food delivery in an animal may cease to elicit dopamine signalling, whereas a conditioned cue previously paired with food delivery still increases dopamine cell firing. In the case of cocaine addiction, every administration of cocaine increases dopamine signalling and thereby promotes the formation of new associations between the drug and the environment (Kalivas and O'Brien, 2008). Accordingly, cocaine addiction can be seen as a pathological form of learning. This concept is of utmost importance in cocaine addiction and may partially explain how drug-associated cues can induce craving and increase the risk for relapse. In line with preclinical studies, a human PET study revealed significantly increased dopamine in the dorsal striatum in chronic cocaine users after they watched a cocaine-cue video with scenes of preparing and using cocaine compared to when they watched a neutral video portraying nature scenes. Moreover, higher increases of dopamine in the dorsal striatum were associated with the subjective experience of cocaine craving and addiction severity (Volkow et al., 2006).

In sum, initially cocaine use particularly increases dopamine release in the ventral striatum to signal reward and to imbue an event with salience, while after repeated administration leading to the development of habits that primarily involve the dorsal striatum, a shift occurs from the drug to conditioned stimuli to elicit dopamine release. Moreover, in chronic cocaine users conditioned cues lead to larger increases of dopamine than cocaine administration, indicating that drug-associated cues may mainly drive dopamine signalling and thereby the motivation to continuously administer cocaine (Volkow et al., 2011).

1.6.3 The role of dopamine in inhibitory control

A further role partially ascribed to dopaminergic signalling is the ability to successfully inhibit prepotent responses, which has been associated with addiction vulnerability by influencing the capacity to abstain from drugs (Volkow et al., 2006). The aforementioned association of reduced striatal D_2 receptor availability with lower metabolism in regions implicated in salience attribution, inhibitory control, emotion regulation, and decision-making (OFC, ACC, DLPFC) has further been hypothesized to reflect insufficient regulation of these cortical regions by dopamine, to underlie the

enhanced motivational salience of cocaine, and to contribute to the inability to control cocaine intake resulting in compulsive drug use (Volkow et al., 2011). However, data from our lab suggest that specifically reward-related impulsivity is compromised in cocaine users (see study 3 in section 5), but not the more basal inhibitory motor control (Vonmoos et al., submitted).

1.6.4 The role of dopamine in motivation

Dopamine also modulates motivational processes by regulating a widespread brain network involving the NAcc, ACC, OFC, DLPFC, amygdala, dorsal striatum, and ventral pallidum (Salamone et al., 2007). The enhanced motivation to obtain drugs in spite of harmful consequences and the exhibition of apathy during non-drug related activities are the core features of addiction (Volkow et al., 2003; Volkow and Li, 2005). Imaging studies in addicted cocaine users have provided evidence for increased prefrontal brain activation patterns upon the presentation of conditioned, craving-inducing cues, in contrast to the decreased prefrontal activity observed in detoxified cocaine users in absence of drug cues or the drug itself (for review see Goldstein and Volkow, 2011). Notably, intravenous administration of methylphenidate increased brain metabolism in the ventral ACC and medial OFC in individuals addicted to cocaine, while the metabolism in these brain regions decreased in non-addicted individuals (Volkow et al., 2005). Taken together, these findings indicate that prefrontal regions only become activated in addicted individuals upon drug use or stimulation with drug-associated cues, which in turn may trigger craving and eventually result in enhanced desire/motivation to obtain cocaine (Volkow et al., 2011).

1.6.5 The role of dopamine in cognition and colour vision

The central dopamine system holds a pivotal role in the mediation of PFC function (Braskie et al., 2008; Nieoullon, 2002; Vernaleken et al., 2007), which is crucial for attention, working memory, and executive functions (Benton, 1994). Consequently, neuropsychological impairment has been consistently reported for cocaine-dependent subjects across several areas of cognitive functioning, including attention, executive function, verbal learning, and memory (Fernandez-Serrano et al., 2009; Goldstein et al., 2004; Kelley et al., 2005; Woicik et al., 2009).

Interestingly, dopamine also exists in high concentrations in the retina (Bodis-Wollner and Tzelepi, 1998; Dowling, 1990; Witkovsky, 2004) where it may be involved in chromatic processing by modulating horizontal cell functioning and the cone-horizontal cell connectivity (Ahnelt and Kolb, 1994; Djamgoz et al., 1997). Indeed, abnormal colour discrimination along the tritan (blue-hue) axis and decreased blue cone b-wave electroretinogram (ERG) amplitudes that were associated with stronger cocaine craving have been observed in chronic cocaine users (Desai et al., 1997; Roy et al.,

1996). The authors concluded that blue-yellow colour vision impairment in dependent cocaine users may reflect a central hypodopaminergic state, as previously demonstrated in several molecular imaging studies (Martinez et al., 2007; Volkow et al., 1990, 1997). Therefore, in the first study presented in detail in section 3, we aimed to investigate if colour vision impairment is confined to dependent cocaine users, whether it is specific for dopaminergic stimulants such as cocaine and amphetamine, and if colour vision impairment is related to cognitive functions such as working memory, encoding, and consolidation, potentially reflecting a link between retinal and cerebral brain dopamine function.

1.7 The glutamatergic corticostriatal circuitry in cocaine addiction

Addiction research has primarily focused on the role of the mesocorticolimbic dopamine system due to its involvement in mediating rewarding effects of addictive drugs, however, the enduring vulnerability to relapse accrues from lingering neuroplasticity in the corticostriatal circuitry in which the dopamine axon terminals are embedded (Kalivas, 2009). In the following sections, pertinent preclinical and human neuroimaging findings regarding cocaine-related alterations in the glutamate system shall be reviewed.

1.7.1 General function of the glutamate system

Glutamate is the most ubiquitous excitatory neurotransmitter in the human brain, accounting approximately for 70% of synaptic neurotransmission in the central nervous system (Erecińska and Silver, 1990), and governing a myriad of processes comprising fast and slow excitatory neurotransmission, control of basal neuronal activity, synaptic plasticity, as well as learning and memory (Anwyl, 1999; Nakanishi, 1994; Olive, 2009). Glutamate can bind to three different kinds of ligand-gated ionotropic glutamate receptors (iGluRs) [N-methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor, kainic acid (kainate, KA) receptor] that mediate fast excitatory neurotransmission (Cull-Candy et al., 2001; Cull-Candy et al., 2006; Lerma et al., 1997) and G-protein coupled metabotropic glutamate receptors (mGluRs) that are involved in slower, modulatory neurotransmission. mGluRs can be divided into three groups on the basis of their second messenger coupling and ligand sensitivity (group I receptors: mGluR1/5 receptors; group II receptors: mGluR2/3; group III receptors: mGluR4/6/7/8) (Javitt, 2004). Extracellular glutamate is cleared by excitatory amino acid transporters (EAATs) that are primarily located on glial cells and prevent excessive accumulation of extracellular glutamate that can lead to excitotoxicity (Olive, 2009; Shigeri et al., 2004). Subsequently, astrocytes convert glutamate to

glutamine and release it back to the extracellular environment, where glutamate is taken up by neurons and converted back to glutamate (Featherstone, 2010). In addition, the cystine-glutamate exchanger (xc^-) transports glutamate from within glial cells to the extracellular space (McBean, 2002; Melendez et al., 2005; Moran et al., 2005; Shigeri et al., 2004).

1.7.2 Enduring glutamatergic neuroplasticity may promote vulnerability to relapse

Accumulating evidence implies that enduring neuroplasticity of the glutamatergic corticostriatal circuitry may be responsible for the persisting vulnerability to craving, relapse-related behaviours, and maintenance of drug addiction. Indeed, glutamatergic projections from the PFC and allocortex (e.g., amygdala, hippocampus) into the striatal motor/habit circuit, including the NAcc, appear to be crucial for executing learned behaviours and the ability to adapt these habitual behaviours if they are no longer useful (Kalivas and Volkow, 2005). Importantly, with repeated execution of well-established behaviour, corticofugal glutamte projections from the PFC and amygdala into the NAcc become less important, while glutamatergic projections from sensory motor cortical areas to the dorsal striatum are strengthened (Everitt and Robbins, 2005). Accordingly, in cocaine addiction, a shift from declarative behaviour relying on prefrontal executive functions to habitual behaviour involving the cortico-striato-thalamic motor circuitry and procedural memories takes place, accounting for the loss of control in regulating drug intake and compulsive relapses (Everitt and Robbins, 2005; Kalivas and O'Brien, 2008).

Preclinical animal models have demonstrated that glutamatergic projections from the PFC to the NAcc appear to be critically implicated in cue-, stress- or cocaine-primed drug reinstatement and drug-seeking behaviour (Kalivas and Volkow, 2005; Kalivas, 2009; McFarland et al., 2003). Studies consistently provided evidence that chronic self-administration of cocaine results in decreased basal levels of non-synaptic extracellular glutamate in the NAcc core in rats (Baker et al., 2003; Kalivas and Brady, 2012; Kalivas and Volkow, 2005; Madayag et al., 2007; McFarland et al., 2003). In contrast, after withdrawal, stimulation with a priming dose of cocaine, drug-related stimuli or external stressors can trigger the release of large amounts of glutamate in the NAcc, which in turn can influence plasticity via binding to postsynaptic receptor proteins (Grover et al., 1999; Malenka and Bear, 2004; McFarland et al., 2003; Moussawi et al., 2009; Pierce et al., 1996; Wu et al., 2004). The explanatory value of this preclinical model is especially intriguing, as it seems to concur with findings from human studies.

Clinical neuroimaging studies have yielded compelling evidence that chronic cocaine users manifest widespread PFC hypoactivity during withdrawal as measured by lower glucose metabolism and blood flow (Volkow et al., 1988, 1991, 1992) and the inability to control drug-seeking has been ascribed to disrupted control of the PFC over subcortical limbic and motor brain regions (Goldstein

and Volkow, 2011) (also see section 1.5 and 1.6). Perhaps in line with these findings, chronic cocaine users exhibited significantly lower glutamate levels in the ACC compared to controls in a MRS study (Yang et al., 2009). Analogous to the aforementioned preclinical studies, exposure to cocaine-associated cues markedly increases PFC activity (including the ACC and ventral orbital cortices) in chronic cocaine users, which has been associated with the subjective intensity of cocaine craving, likely increasing the occurrence of relapse (for review, Goldstein and Volkow, 2002, 2011).

In sum, accumulating evidence suggests that enduring neuroplasticity of the cortical glutamate circuitry may be responsible for the persisting vulnerability to craving, relapse-related behaviours, and maintenance of drug addiction. Animal models have contributed tremendously to shed light on enduring cellular neuroplasticity and have led to the formulation of the glutamate hypothesis of cocaine addiction that shall be delineated in the next section.

1.7.3 The glutamate hypothesis of cocaine addiction

The term glutamate homeostasis refers to balanced regulation of extracellular non-synaptic and synaptic glutamate. In order to maintain homeostasis, glial and synaptic glutamate release and elimination have to be tightly regulated. Impaired glutamate homeostasis impacts synaptic activity and plasticity by modulating glutamate access to iGluRs and mGluRs (Kalivas 2009).

The decreased extracellular basal glutamate levels observed in the rat NAcc core (~50%) following chronic exposure and withdrawal from cocaine are linked to down-regulated expression of the xc^- (Madayag et al., 2007; Trantham-Davidson et al., 2012). xc^- is predominantly expressed on glial cells and along with glutamate transporters (GLT1; also primarily located on glial cells in vicinity to the synaptic cleft) holds a critical role in maintaining extracellular glutamate (~60% of basal extracellular glutamate is derived from xc^- function) (Baker et al., 2002; Featherstone, 2010). Lower basal glutamate levels after cocaine self-administration reduce the tone on presynaptically located glutamate release-regulating mGluRs of type 2/3, which in turn results in reduced inhibition and thus in increased synaptic glutamate release (Baker et al., 2002; Moran et al., 2006). Consequently, this affects stimulation of postsynaptically located iGluRs and mGluRs, which finally influences synaptic activity and plasticity via long-term potentiation (LTP) and long-term depression (LTD), thereby shaping learning and memory processes (Kalivas, 2009). Upon drug-, stress- or cue-induced reinstatement of drug-seeking in rats large amounts of glutamate are released into the NAcc core (synaptic overflow). This is partly due to sensitization, increased glutamate release due to reduced mGluR2/3 inhibition, but also the down-regulation of GLT-1 further influences synaptic glutamate transmission by reduced elimination of glutamate from the extracellular space (Knackstedt et al., 2010; Madayag et al., 2007; McFarland et al., 2003, 2004; Miguens et al., 2008; Pendyam et al., 2009) (Fig. 2).

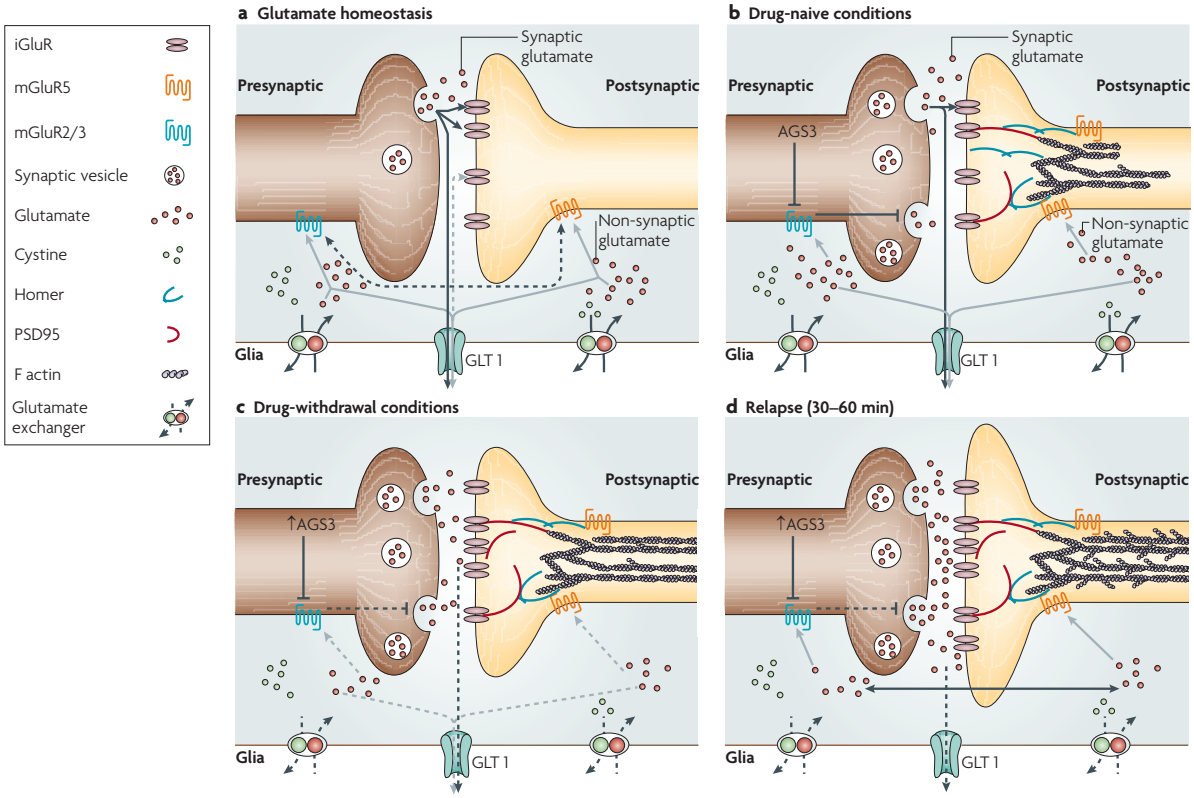

Fig. 2. Neuroadaptations produced by the effects of chronic cocaine administration on protein content and function in excitatory synapses in the nucleus accumbens. **a)** Glutamate uptake through glutamate transporter 1 (GLT1) limits the access of synaptically released glutamate to the non-synaptic extracellular space and metabotropic glutamate receptors (mGluRs), and limits the access of glutamate released non-synaptically (through cystine–glutamate exchange) to the synaptic cleft and ionotropic glutamate receptors (iGluRs). As a result, glutamate derived from cystine–glutamate exchange does not readily stimulate synaptic iGluRs. Solid lines illustrate the physiological interactions under basal conditions; dotted lines illustrate minor interactions. **b)** In control (that is, drug-naive) conditions, glutamate homeostasis maintains concentrations of extracellular synaptic and non-synaptic glutamate that permit synaptic grading and the induction of long-term potentiation and long-term depression. **c)** Changes that occur after withdrawal from chronic cocaine administration. In this condition, reduced cystine–glutamate exchange results in a decrease in basal extrasynaptic glutamate levels. In addition, reduced tone on mGluR2 and mGluR3 (mGluR2/3) results in an increase in synaptically released glutamate. In the postsynaptic neuron, filamentous (F) actin, AMPA (α-amino-3-hydroxy-5-methyl-4- isoxazole propionic acid) iGluRs and postsynaptic density protein 95 (PSD95) levels are elevated, whereas Homer levels are reduced. **d)** Changes in glutamate homeostasis and protein content in the nucleus accumbens seen 30 to 60 min following the induction of cocaine seeking by an acute cocaine injection (relapse). Synaptic glutamate levels are increased owing to enhanced release and to reduced elimination of glutamate from the extracellular space by GLT1. In addition, the surface expression of AMPA iGluRs is elevated and spine head diameter is further increased. Reprinted by permission from Macmillan Publishers Ltd: Kalivas, 2009, Nature Reviews Neuroscience 10(8): 561-572, copyright 2009.

1.7.4 Specific cocaine-induced alterations in the glutamate system

After chronic contingent and non-contingent cocaine administration in rats, a number of alterations occur in the morphology of dendritic spines as well as in the function and expression of ionotropic and metabotropic glutamate receptors in the NAcc, which are subject to change again upon acute cocaine administration (Kalivas, 2009).

Influence on ionotropic glutamate receptors. For instance, after chronic contingent and non-contingent cocaine administration in rats, rapid increases in head diameter of dendritic spines were observed 45 minutes after acute cocaine administration, which is consistent with increased AMPA receptor insertion (Anderson et al., 2008), while 120 minutes after cocaine injection the spine diameter was markedly reduced, consistent with AMPA receptor internalization (Boudreau et al., 2007).

Influence on metabotropic glutamate receptors. As previously mentioned, chronic cocaine administration is associated with down-regulation of the presynaptically located group II mGluR2/3s, leading to increased synaptic release of glutamate due to weakened inhibition provided by mGluR2/3s (Baker et al., 2002; Moran et al., 2006). In addition, although not entirely consistent in all preclinical studies, down-regulation also occurs in group I mGluR1/5s and their intracellular binding proteins Homer 1b/c (Ary and Szumlinski, 2007; Ben-Shahar et al., 2009; Fourgeaud et al., 2004; Ghasemzadeh et al., 1999, 2009; Hao et al., 2010; Mitrano et al., 2008; Swanson et al., 2001). Down-regulation of mGluR2/3 seems to promote drug seeking as pharmacological agonists result in attenuated reinstatement of cocaine seeking, while the down-regulation of mGluR1/5 and Homer appears to be a compensatory mechanism because pharmacological antagonism or deletion of the mGluR5 attenuates cocaine seeking (Kalivas, 2009). Interestingly, extinction training has also been associated with a marked decrease in mGluR5 expression and elevated levels of Homer proteins, possibly providing further support for a compensatory adaptation, which may inhibit relapse (Kalivas and Volkow, 2011). As the mGluR5 was subject of the second study of the present thesis, more detailed information regarding its function in addiction will be provided in the next section.

Metaplasticity. The down-regulation of cystine-glutamate exchange observed after extended withdrawal from chronic cocaine administration and the resulting loss of tone on mGluRs has been associated with bidirectional loss of synaptic plasticity in the NAcc. On the one hand, an increase in AMPA/NMDA ratio occurs, indicating LTP (Conrad et al., 2008; Kourrich et al., 2007), on the other hand also attenuated LTD has been observed in the NAcc core (Martin et al., 2006). These disruptions in synaptic plasticity may have important implications for learning and memory processes associated with the maintenance of addiction and the inability to form new adaptive behaviours to inhibit relapse.

1.7.5 The metabotropic glutamate receptor 5 in cocaine addiction

The mGluR of type 5 (mGluR5) has gained growing attention in addiction research due to its high expression in corticolimbic regions implicated in drug addiction including the medial PFC, OFC, cingulate, striatum, amygdala, and hippocampus (Abe et al., 1992) and involvement in drug-seeking behaviour and extinction learning in animals withdrawn after cocaine self-administration (for reviews, Bellone and Mameli, 2012; Duncan and Lawrence, 2012; Kenny and Markou, 2004; Olive, 2010). mGluR5s are mainly located postsynaptically on the perisynaptic annulus of dendritic spines of neurons and on glial cells (Castillo et al., 2010; Lujan et al., 1996, 1997; Tallaksen-Greene et al., 1998). Activation of group I mGluRs leads to increased hydrolysis of membrane phosphoinositide via phospholipase C activation, producing diacylglycerol, which in turn activates protein kinase C (PKC) and inositol-1,4,5-triphosphate that induces release of Ca^2 from intracellular stores and then stimulates PKC (Abe et al., 1992; Kew and Kemp, 2005; Masu et al., 1991). mGluR5s are involved in slow glutamate-mediated neurotransmission and have a primarily modulatory role (Hovelsø et al., 2012). Although the exact function of mGluR5s in addiction is not entirely understood, they appear to regulate drug-induced synaptic plasticity in brain regions that are thought to be involved in shaping learning and memory processes, partly through their structural and functional interaction with NMDA receptors (Collett and Collingridge, 2004; Nicoletti et al., 2011), and have directly been linked to drug-seeking behaviour and extinction learning in animals withdrawn after cocaine self-administration (for review, Bellone and Mameli, 2012; Duncan and Lawrence, 2012; Kenny and Markou, 2004; Olive, 2010). For instance, mGluR5 null mutant mice did not self-administer cocaine or exhibit increased locomotor activity after cocaine treatment (Chiamulera et al., 2001) and self-administration and reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin is attenuated by mGluR5 antagonists (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Kenny et al., 2005; Kumaresan et al., 2009; Lee et al., 2005; Martin-Fardon et al., 2009; Paterson and Markou, 2005; Paterson et al., 2003; Platt et al., 2008; van der Kam et al., 2007). Moreover, it has been suggested that genetic variations in mGluR5s, resulting in decreased mGluR5-mediated neurotransmission, may render individuals less sensitive to the reinforcing effects of cocaine and nicotine, as well as aversive states during withdrawal as the chromosomal region 11q14, on which the GRM5 gene is located, has been associated with habitual smoking behaviour (Bierut et al., 2004; Stoker et al., 2012). Several explanations have been proposed how mGluR5s modulate cocaine-mediated behaviours and which mechanisms underlie the effectiveness of pharmacological antagonists, however, more research is required to confirm them. One suggestion was that in the NAcc interactions of mGluR5s with the Homer family, post-synaptic scaffolding proteins influencing mGluR5 trafficking and signal transduction, may contribute to AMPA receptor trafficking changes (Kumaresan et al., 2009). In addition, it was shown that a single exposure to cocaine can lead to

reduced expression of mGluR5s in rats, which may transiently impair mGluR5-dependent LTD mediated via activation of cannabinoid-1 receptors (Fourgeaud et al., 2004; Luscher and Huber, 2010).

1.7.6 Medication development

The growing understanding of these molecular glutamatergic alterations has raised the exciting possibility to develop novel pharmacological compounds to restore the glutamate homeostasis by targeting different underlying mechanisms. Some medications have already entered clinical trials for other psychiatric disorders and it is intended to evaluate their ability to contribute to drug addiction treatment as well (Haile et al., 2012; Javitt et al., 2012; Kalivas and Volkow, 2011; Olive et al., 2012; Olive, 2009; Reissner and Kalivas, 2010). Much effort is currently being undertaken to develop pharmacological compounds with the ability to block mGluR5s, which might be one efficacious way to treat stimulant-addiction. However, if mGluR5 function and expression is altered in human cocaine users has not been investigated so far. Therefore, in the second study of the present thesis, mGluR5 availability in cocaine users was quantified and compared to drug-naïve healthy controls in a PET study (see section 4).

1.8 Behavioural consequences of long-term cocaine exposure – Impaired decision-making

As reviewed in section 1.5, it has been postulated that disrupted PFC function in cocaine users accounts for the loss of willed behaviours and the inability to adaptively regulate habitual drug seeking behaviour, excessive salience attribution to cocaine or cocaine-related cues, cognitive deficits, and decision-making impairment (Goldstein and Volkow, 2002, 2011). Indeed, maladaptive decision-making is a hallmark of cocaine addiction that is well captured by the paradox that addicted cocaine users compulsively seek and take the drug even at the expense of harmful financial, health-related, or social consequences (APA, 2000; Koob, 2009b). Converging evidence from human neuroimaging and lesion studies as well as preclinical physiological experiments have revealed that particularly the OFC and ventromedial PFC hold a pivotal role in regulating decision-making (Bolla et al., 2003; Dom et al., 2005; Koob and Volkow, 2010; Lucantonio et al., 2012; Olausson et al., 2007; Schoenbaum et al., 2006; Winstanley, 2007).

1.8.1 The role of the orbitofrontal cortex in decision-making

The term decision-making describes the ability to choose an optimal course of action from multiple alternatives and requires constant updating and integrating of information about the value of present and potential actions as well as future states pertaining to current needs (Fellows, 2004; Lucantonio et al., 2012). Different lines of evidence revealed that the OFC mediates decision-making and elucidated specific functions of the OFC. The first indication came from patients with lesions in the OFC that were associated with a unique pattern of deficits. These patients had intact cognitive abilities but showed severe difficulties in successfully navigating everyday life. Anecdotal reports about a patient called Elliott, who presented with bilateral damage to the OFC after removal of a brain tumour, gave account of dramatic behavioural changes. Elliot quit his job, divorced his wife, lost contact with friends and family, lost a large amount of money to a scam artist, and married a prostitute he had only known for a month within months after undergoing surgery. Moreover, even a simple decision such as where to eat, would take him hours (Damasio, 1994; Eslinger and Damasio, 1985). A different line of evidence stems from seminal experiments in OFC-lesioned rats and monkeys as well as comparable fMRI tasks in humans implicating the OFC in guiding behaviour on the basis of information about expected appetitive or aversive outcomes (assigning salience or value to stimuli) and in learning in response to changes in these outcomes (reversal learning) (Fellows and Farah, 2003; Gallagher et al., 1999; Gottfried et al., 2003; Izquierdo et al., 2004; Schoenbaum and Shaham, 2008). For instance, in one experiment a neutral cue was paired with the delivery of an appetitive food reward in hungry rats. Subsequently, the food reward was paired with lithium chloride, which induces nausea and aversion to the food, resulting in devaluation of the food reward (Gallagher et al., 1999). Although OFC-lesioned rats showed normal conditioning and devaluation of the food outcome, they continued to pursue the devalued outcome. Moreover, OFC-lesioned monkeys were unable to modify their behaviour regarding stimulus-reward reversals and perseverated to choose an object that was previously rewarded but failed to learn that the previously unrewarded object now lead to the reward (Mishkin, 1964). Finally, it is noteworthy that the OFC cortex receives and integrates information from all sensory modalities, and is connected to the NAcc, the hypothalamus, and the limbic system including the amygdala, hippocampus, and cingulate gyrus (Carmichael and Price, 1995; Cavada et al., 2000), suggesting that the OFC may be implicated in calculating the value of a reward outcome by integrating sensory and affective information (Wallis, 2007).

1.8.2 Orbitofrontal cortex dysfunction associated with cocaine use

Structural and functional imaging studies consistently reported decreased grey matter density and reduced metabolism in the OFC, ACC, and the DLPFC, as well as decreased frontal white matter in dependent human cocaine users (Ersche et al., 2011; Franklin et al., 2002; Lucantonio et al., 2012; Lyoo et al., 2004; Matochik et al., 2003; Sim et al., 2007). Notably, the gray matter reduction observed in the OFC in cocaine users correlated with the duration and compulsivity of cocaine use. However, these data cannot conclusively answer if the structural and functional alterations in the OFC are cocaine-induced or may represent a pre-existing condition. More conclusive evidence stems from a growing number of preclinical neurophysiological studies demonstrating performance deficits after exposure to cocaine in reversal learning, the devaluation task described in the previous section, and the delay discounting (DD) paradigm where animals have to learn to forgo an immediate smaller reward in favour of a larger delayed reward (Calu et al., 2007; Mendez et al., 2010; Porter et al., 2011; Schoenbaum et al., 2004). Findings with regard to decision-making deficits in human cocaine users shall be elaborated on in the next two sections.

1.8.3 Classical, non-social decision-making

A commonly used behavioural measure of decision-making is the *Iowa Gambling Task* (IGT). The IGT factors several different aspects of decision-making, including uncertainty, risk, and processing of reward and punishment contingencies (Bechara et al., 1994, 2000a, 2000b). A PET study measuring glucose metabolism in chronic cocaine users who had been abstinent for 25 days, revealed that activation in the OFC was directly related to better IGT performance in the cocaine user group and the control group. Moreover, in the cocaine user group, OFC activation was negatively related to amount of cocaine used. In addition, numerous behavioural studies demonstrated that dependent cocaine users were more likely to choose disadvantageous high-risk card decks resulting in net loss over time than advantageous card decks in comparison to controls (Bechara and Damasio, 2002; Kjome et al., 2010; Verdejo-Garcia et al., 2007). These results have been interpreted in such a way that chronic cocaine users experience "*myopia for the future*" indicating that they fail to incorporate ongoing feedback to guide future outcomes and instead process decisions only with regard to their immediate reward availability.

A further aspect of decision-making, in which dependent cocaine users have consistently been shown to be impaired, is intertemporal choice or *Delay Discounting*. When presented with a choice between a smaller immediately available or a larger but delayed reward, dependent cocaine users discounted delayed rewards more steeply than controls (Bickel et al., 2007, 2011; Coffey et al., 2003; Heilet al., 2006; Kirby and Petry, 2004; MacKillop et al., 2011; Mendez et al., 2010); in other words

they exhibit an inability to wait for delayed rewards, which has also been referred to as "impulsive choice" (Ainslie, 1975; Rachlin and Green, 1972). It is noteworthy that steeper discounting rates in the general population have been associated with negative outcomes in the financial, academic, and health domain (Mischel et al., 2011) and poor treatment response in cocaine dependent individuals (Washio et al., 2011).

In sum, decision-making deficits have concordantly been demonstrated in dependent cocaine users. However, if these deficits even occur in recreational, non-dependent cocaine users has not been investigated and was subject of the third study of the present thesis (section 5).

1.8.4 Social decision-making

As we live in complex, continuously changing social environments, decision-making often takes place in the form of social interaction. Decisions embedded in a social context not only affect one-self but also others and are heavily influenced by self and other-regarding preferences (Fehr and Camerer, 2007). Social decision-making encompasses multiple facets (e.g., trust, cooperation, un-/fairness, altruism, norm-abiding decision-making, punishment, social learning, and competitive social interactions) and is orchestrated in various brain regions including the PFC and particularly the ventromedial and orbital PFC, but also the ACC, anterior insula, ventral striatum, and amygdala (Rilling and Sanfey, 2011). Because cocaine users exhibit alterations in most of these brain areas (Goldstein and Volkow, 2011), the notion that they may also display deficits in social decision-making seems probable.

Adequate social cognitive abilities (e.g., theory of mind, empathy, emotion recognition) are a prerequisite for social decision-making and it is noteworthy that social cognition has been shown to strongly impact the development, course, and outcome of psychiatric diseases (Couture et al., 2006) and may also affect the course of dependence and treatment success in cocaine abusers (Homer et al., 2008). Although social interaction in cocaine users has not been studied in an experimental setting, there are a number of findings pointing to deficient social cognitive abilities in cocaine users. For instance, cocaine users feature a 22-fold increased risk for an antisocial personality disorder and clinical reports have given account of egocentrism and blunted emotion (Rounsaville, 2004). Moreover, in a study with crack cocaine-dependent individuals decision-making deficits were associated with real-life social dysfunction. However, social dysfunction was solely based on a self-report scale (Cunha et al., 2011).

Advances in the burgeoning field of neuroeconomics and game-theoretic approaches have provided the unique opportunity to quantify social decision-making characteristics during social interaction situations in psychiatric disorders (Hasler, 2011; Kishida et al., 2010). Therefore, considering the high relevance of adequate social interaction for everyday life situations, a further

goal of the third study was to assess social decision-making in the context of a more naturalistic interaction paradigm aimed at measuring preferences for fairness vs. selfishness in money distribution games with recreational and dependent cocaine users and stimulant-naïve controls (section 5).

1.9 References

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2

OVERVIEW OF ORIGINAL RESEARCH

2.1 Research objectives

Converging evidence from preclinical and human neuroimaging studies has revealed that drug-induced neuroadaptations in the mesocorticolimbic dopamine system and the corticostriatal glutamate circuitry mediate the development and maintenance of cocaine addiction in combination with predisposing genetic, developmental, and environmental factors (Kalivas and O'Brien, 2008). Moreover, drug-associated disruption of PFC function, particularly in the ventromedial/orbital parts and the anterior cingulate gyrus, have been proposed to underlie the loss of control exhibited as compulsive drug use behaviour, cognitive deficits, and decision-making impairment in dependent cocaine users (Goldstein and Volkow, 2011). Antecedent findings leave a number of important issues unresolved. Therefore, the key objectives for each study and the deduced hypotheses shall be briefly outlined:

Study 1: Blue-yellow colour vision impairment and cognitive deficits in occasional and dependent stimulant users

Prior studies have consistently demonstrated specific blue-yellow colour vision impairment in dependent cocaine-withdrawn users and it has been postulated that cocaine-induced changes in retinal dopamine might be responsible (Desai et al., 1997; Roy et al., 2003). However, it has not been examined if colour vision impairment is confined to chronic cocaine users, whether it is specific for dopaminergic stimulants, and if it is related to cognitive performance.

Hypotheses:

1) *We expect that compared to controls even recreational, non-dependent cocaine users may manifest more frequent, specific blue-yellow colour vision impairment, but that these impairments are less pronounced than in dependent cocaine users.*
2) *We hypothesize that colour vision impairment may be specifically linked to drugs altering the dopamine system (cocaine) and not the serotonin system (MDMA).*
3) *We suppose that worse colour discrimination indices will be associated with longer and more pronounced cocaine use.*
4) *We assume a potential link between colour vision impairment and cognitive deficits in cocaine users.*

Study 2: Nicotine but not cocaine use is associated with decreased cerebral metabotropic glutamate receptor 5 density in humans

Preclinical findings indicate that long-lasting adaptations in the glutamatergic corticostriatal circuitry may be responsible for the enduring vulnerability to relapse and maintenance of cocaine addiction (Kalivas, 2009). In particular, the metabotropic glutamate receptor type 5 (mGluR5) has directly been implicated in drug reinstatement in animal models and has gained interest as a potential target for new pharmacotherapies due to the fact that mGluR5 antagonists attenuated self-administration of cocaine and other drugs of abuse in rats (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Paterson and Markou, 2005; Paterson et al. 2003; Platt et al., 2008; van der Kam et al. 2007). Most preclinical studies have shown that mGluR5s become down-regulated in the NAcc core after chronic cocaine administration in rats (McFarland et al., 2003; Pierce et al., 1996). Moreover, a study using MRS in human crack cocaine users reported lower free glutamate levels in the ACC (Yang et al., 2009). To what extent preclinical findings are comparable to human cocaine users has not been investigated to date.

Hypotheses:

1) *We expect that cocaine users may exhibit decreased mGluR5 availability compared to drug-naïve controls in the striatum (NAcc) and likely also in the ACC and other corticolimbic, addiction-related brain areas.*
2) *Lower mGluR5 availability in cocaine users is expected to correlate with cocaine use patterns.*

Study 3: Opportunism and immediacy bias in recreational and dependent cocaine users

Behavioural studies have concordantly shown that dependent cocaine users exhibit decision-making deficits in the IGT that requires the incorporation of positive and negative feedback regarding reward/punishment contingencies, and the DD paradigm where chronic cocaine users preferred immediate smaller rewards over larger delayed rewards (Kirby and Petry, 2004; Verdejo-Garcia et al., 2007). If decision-making is compromised to a similar extent in non-addicted, recreational cocaine users and if these deficits extend to social decision-making has not been investigated thus far. Therefore, we intended to compare the performance of recreational and dependent cocaine users in non-social (i.e., IGT, DD) and interactive social decision-making (i.e., Distribution Game, Dictator Game) tasks with the decision-making performance of drug-naïve controls.

Hypotheses:

1) *We hypothesize that recreational cocaine users show an intermediate performance in non-social decision-making between controls and dependent cocaine users.*
2) *We further expect that both recreational but in particular dependent cocaine users will allocate money in a more selfish manner in the social interaction paradigms compared to controls.*
3) *We suppose that cumulative cocaine use and duration of cocaine use will correlate with non-social and social decision-making performance.*

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3

BLUE-YELLOW COLOUR VISION IMPAIRMENT AND COGNITIVE DEFICITS IN OCCASIONAL AND DEPENDENT STIMULANT USERS

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LMH collected and analyzed the data, interpreted the data, and wrote the manuscript. BBQ designed the study, helped to interpret the data and revised the first draft of the manuscript. MW was involved in designing the study and revised the first draft of the manuscript. KHP and DJ contributed to data acquisition and/or revised the first draft of the manuscript.

3.1 Abstract

Background. Specific blue-yellow colour vision impairment has been reported in cocaine-dependent users and it was postulated that drug-induced changes in retinal dopamine neurotransmission are responsible. However, it is unclear whether these changes are confined to chronic cocaine users, whether they are specific for dopaminergic stimulants such as cocaine and amphetamine, and whether they are related to cognitive functions such as working memory, encoding, and consolidation.

Method. In 47 occasional and 29 dependent cocaine users, 23 MDMA users, and 47 stimulant-naïve controls, colour vision discrimination was measured with the Lanthony Desaturated Panel D-15 Test and memory performance with the Auditory Verbal Learning Test.

Results. Both occasional and dependent cocaine users showed higher Colour Confusion Indices than controls. Users of the serotonergic stimulant MDMA (26%), occasional (30%) and dependent cocaine users (34%) exhibited more frequent blue-yellow colour vision disorders compared to controls (9%). Inferior performance of MDMA users was caused by a subgroup with high amphetamine co-use (55%), while MDMA use alone was not associated with decreased blue-yellow discrimination (0%). Cognitive performance was worse in cocaine users with colour vision disorder compared to users and controls with intact colour vision and both colour vision impairment and cognitive deficits were related to cocaine use.

Conclusions. Already occasional cocaine and amphetamine use might induce blue-yellow colour vision impairment, whereas the serotonergic stimulant MDMA does not impair colour vision. The association between colour vision impairment and cognitive deficits in cocaine users may reflect that retinal and cerebral dopamine alterations are linked to a certain degree.

3.2 Introduction

The psychostimulant cocaine exerts its reinforcing effect primarily via inhibiting the uptake of dopamine in the nucleus accumbens and additional structures of the reward system (Ritz et al., 1987). Numerous imaging studies with dependent cocaine users have provided evidence for blunted dopamine neurotransmission (Martinez et al., 2004, 2007, 2009, 2011; Volkow et al., 1993, 1997; Wu et al., 1997) and abnormalities in cerebral glucose metabolism in the prefrontal cortex (PFC) and further limbic areas (Bolla et al., 2004; Volkow et al., 1992), leading to widespread motivational, cognitive, behavioral, and psychiatric consequences (Bolla et al., 2004). The central dopamine system holds a pivotal role in the mediation of PFC function (Braskie et al., 2008; Nieoullon et al., 2002; Vernaleken et al., 2007), which is crucial for attention, working memory, and executive functions (Benton et al., 1994). Additionally, it was recently demonstrated that dopamine neurotransmission is critically involved in encoding, consolidation, and retrieval of declarative memory (Breitenstein et al., 2006*a*, *b*; Morris et al., 2003; Whiting et al., 2007, 2008). Consequently, neuropsychological impairment has been consistently reported for cocaine-dependent subjects across several areas of cognitive functioning, including attention and executive function, verbal learning and memory (Fernandez-Serrano et al., 2009; Goldstein et al., 2004; Kelley et al., 2005; Woicik et al., 2009).

Dopamine also exists in high concentrations in the retina, where it is localized in the amacrine and interplexiform retinal cells (Bodis-Wollner and Tzelepi, 1998; Dowling, 1990; Witkovsky, 2004). Dopamine acts in a complex fashion on visual information processing and is involved in the regulation of lateral inhibition, center-surround antagonism, and light adaptation, promoting the light-driven cone input and reducing the rod input (Brandies and Yehuda, 2008; Sannita, 1995; Witkovsky, 2004). Moreover, dopamine may also be involved in chromatic processing by modulating horizontal cell functioning and the cone-horizontal cell connectivity (Ahnelt and Kolb, 1994; Djamgoz et al., 1997). Indeed, abnormal colour discrimination has been reported for several neuropsychiatric conditions featuring altered dopaminergic mechanisms, such as Parkinson's and Huntington's disease, Tourette syndrome, and attention deficit hyperactivity disorder (Djamgoz et al., 1997; Melun et al., 2001; Paulus et al., 1993; Pieri et al., 2000; Sartucci et al., 2003; Tannock et al., 2006). These conditions were mainly associated with specific colour vision impairment (CVI) along the tritan (blue-hue) axis, which is usually not affected in congenital CVI. Chronic cocaine use has also been associated with blue-yellow CVI: Desai et al. (1997) reported that more than 48% of dependent cocaine-withdrawn patients exhibited CVI along the tritan axis in comparison to 6.5% of the control subjects. Furthermore, significantly decreased blue cone b-wave electroretinogram (ERG) amplitudes were discovered in recently withdrawn cocaine-dependent patients, and lower blue cone b-waves were associated with stronger cocaine craving in these patients (Roy et al. 1996, 1997*a*). A

subsequent longitudinal investigation revealed that the CVI persisted together with cocaine craving over at least eight weeks (Roy et al., 1997*b*). The latest study of Roy et al. (2003) showed that patients with a reduced blue cone b-wave amplitude exhibited significantly lower concentrations of the major dopamine metabolite homovanillic acid (HVA) in the cerebrospinal fluid (CSF). The authors concluded that blue-yellow CVI in cocaine-dependent subjects reflects a central hypodopaminergic state, as it was previously demonstrated in several molecular imaging studies (Martinez et al., 2007; Volkow et al., 1990, 1997). However, it was not investigated so far if CVI in cocaine users is correlated with behavioural or cognitive deficits, which could further indicate a potential association with frontostriatal dopamine dysfunction (Backman and Farde, 2001). Prior studies primarily focused on chronic/dependent cocaine users and it remains unclear if already occasional cocaine users show CVI. Neither has it been examined if other stimulant drugs with different mechanisms of action, such as MDMA, also induce CVI. The substituted amphetamine derivate MDMA mainly acts at the serotonin system and may ultimately induce serotonergic terminal loss but has not been shown to cause long-term alterations of the dopamine system (Capela et al., 2009; Green et al., 2003).

In the present study, we aimed to clarify at which degree of cocaine exposure CVI occurs and how specific it is for cocaine use. Moreover, we investigated the potential association between CVI and cognitive alterations in stimulant users. 1) We hypothesized that colour vision, and in particular, blue-yellow colour vision is specifically impaired by the use of stimulants altering the dopamine system (cocaine and amphetamine) and not by substances mainly targeting other neurotransmitter systems such as the serotonin releaser MDMA. 2) We expected that cocaine-related CVI would be influenced by different patterns of cocaine use, wherefore we compared occasional, non-dependent cocaine users who had used cocaine over shorter time periods, less frequently and in smaller doses with dependent cocaine users. 3) We reasoned that cocaine users with CVI would also exhibit more pronounced cognitive deficits reflecting alterations of the central dopamine system.

3.3 Materials and methods

3.3.1 Study design and subjects

The sample consisted of 48 occasional cocaine users, 30 dependent cocaine users, 24 MDMA users, and 48 psychostimulant-naïve control subjects (total n=150). Subjects were recruited in Zurich by advertisements in widely read local newspapers, different drug prevention and treatment centers, psychiatric hospitals, internet platforms, and snowball communication. Inclusion criteria were non-dependent cocaine use (1-5 grams per month, DSM-IV criteria of cocaine dependence not met) dependent cocaine use (≥5 grams per month, dependence according to DSM-IV criteria), or

recreational MDMA use (>50 pills lifetime). Abstinence duration of cocaine/MDMA use had to be shorter than 6 month. Participants' age had to be between 18 to 65 years. Exclusion criteria were Axis I or II DSM-IV psychiatric disorders other than cocaine and alcohol abuse/dependence, neurological disorders or head injury, use of opioids or prescription drugs affecting the CNS. Participants had to abstain from illegal substances for a minimum of three days and from alcohol for at least 24 hours. Urine samples were collected to ensure abstinence, whereby six occasional and 13 dependent cocaine users tested positive for cocaine. In order to take effects of acute cocaine use on colour vision into account, additional analyses were carried out to determine whether subjects with positive and negative urine toxicology differed in their colour vision performance. Several subjects of the control group had used cannabis. It was decided not to exclude them from the analyses as cocaine and MDMA users had used cannabis as well. Four subjects (one from each group) were excluded due to congenital CVI in the red-green spectrum. The study was approved by the Cantonal Ethics Committee of Zurich (KEK).

3.3.2 Procedure

Neuropsychological and colour vision assessment was carried out after written informed consent was provided by all participants. Moreover, a *Structured Clinical Interview for DSM-IV Disorders* was carried out by a trained psychologist. To estimate premorbid verbal intelligence the *Mehrfachwahl-Wortschatz-Intelligenztest* (MWT-B) (Lehrl et al., 1999) was applied. Drug use was assessed by means of the *Interview for Psychotropic Drug Consumption*, which has been described in detail in our previous work (Quednow et al., 2004). The brief version of the *Cocaine Craving Questionnaire* (CCQ) was used to assess current cocaine craving (Sussner et al., 2006; Tiffany et al., 1993).

3.3.3 Colour vision assessment and scoring

To assess acquired CVI the *Lanthony Desaturated Panel D-15 test* (LD-15) was used (Lanthony, 1978). The test consists of a fixed reference cap and 15 movable colour caps that have to be arranged in consecutive order. Colours are of low saturation (decreased chroma) and increased lightness. The test was performed under a daylight fluorescent lamp (GTI norm daylight, D65, 6500 K), providing an illumination of 1400 lux. Although no time limit was imposed for test completion, all participants completed the LD-15 within a range of 20 and 560 seconds (mean=102.50, SD±72.38). *Qualitative scoring* is achieved by graphically scoring the test on a template that describes a hue circle based on the placement of the caps in the International Commission on Illumination (CIE) colour space (Wyszecki and Stiles, 1982). Single cap inversions (e.g., 1-3-2-4-…) are classified as minor errors,

whereas cap reversals spanning two or more positions are considered major errors. For each individual, different colour vision error-type patterns (protan [red], deutan [green], and tritan/tetartan [blue-yellow] colour vision deficits) can be derived by drawing lines between consecutive caps. Types of acquired dyschromatopsia relied on Verriest's classification: type I reflects CVI along the red-green axis, type II is a combined impairment of the red-green and blue-yellow axis, type III reflects blue-yellow axis impairment and type IV is diagnosed when no clear pattern can be determined. *Quantitative scoring* is based on the colour scoring method proposed by Geller (2001), who provided a table that yields the Total Colour Distance Score (TCDS) in the CIELAB space. A perfect TCDS score of 56.41 results when all the caps are arranged in consecutive order. A Colour Confusion Index (CCI) error score can be calculated by dividing an individual's actual TCDS score by the ideal score. A CCI value of 1 constitutes the perfect score while higher values indicate CVI.

3.3.4 Cognitive assessment

Verbal learning, declarative memory and executive memory functions were assessed by using a German version (Helmstaedter et al., 2001) of the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964). The RAVLT was chosen because both cocaine and MDMA users have been shown to exhibit marked deficits in verbal learning and declarative memory (Kalechstein et al., 2007; Kelley et al., 2005). The test has been described in detail elsewhere (Quednow et al., 2006). In brief, the RAVLT consists of a word list A containing 15 nouns (learning list), a second list B with 15 different nouns (interference list) and a third list C comprising 50 nouns including all words of list A and B as well as 20 new words that are semantically and phonetically related (recognition list). As dependent variables were assessed: working memory span (supraspan, trial 1), encoding (learning performance, Σ trials 1-5), recall consistency in percent (according to Delis et al. 1987), recall of interference material (list B), recall after interference (trial 6), long-term memory (delayed recall, trial 7 after 30 minutes), consolidation/retrieval (trial 5 minus 7), and adjusted recognition performance [*p(A), p(B)*) Forrester and Geffen, 1991].

3.3.5 Statistical analysis

Statistical analyses were performed with PASW 18.0 (SPSS Inc.). Qualitative data were analyzed by means of frequency analyses (Pearson's Chi^2 test and Fisher's exact test where appropriate) and quantitative data by analyses of variance (ANOVA). For *post hoc* analyses, Sidak corrections were applied. As the assumptions of homoscedasticity and parametric distribution were not met by the variables age, years of education, drug use parameters, and colour discrimination data, these variables were log-transformed (log10) and the constant 1 was added because the data contained 0 values. Log-

transformed values are not reported. Because age, sex, and alcohol/nicotine consumption was shown to have an impact on colour vision (Erb et al., 1999; Jackson and Owsley, 2003; Mergler et al., 1988; Rodriguez-Carmona et al., 2008), and years of education differed between groups, these parameters were introduced as covariates in analyses of covariance (ANCOVA) of the CCI. Age and years of education were introduced as covariates in ANCOVAs of memory performance. Correlation analyses (Pearson's product-moment) and binary logistic regression analyses were conducted to relate drug use parameters to deficits in colour discrimination and cognitive performance. Sensitivity and specificity of the LD-15 were calculated according to Cooper et al. (1979) and ICCVAM (1997). Effect sizes were calculated with G*Power 3.1 (Faul et al., 2007).

3.4 Results

3.4.1 Demographic variables and drug use

Information regarding demography and verbal IQ are displayed in Table 1. Groups did not differ in gender distribution, verbal IQ, and smoking status, while there were significant differences in age and years of education. Dependent cocaine users were significantly older ($p<.001$) and had fewer years of education ($p<.01$-$.001$) than participants from all other groups. However, MDMA users, occasional cocaine users and controls were well matched among all variables. Age and years of education were still introduced as covariates in all analyses.

The drug use pattern of all groups is shown in Table 2. Dependent cocaine users smoked significantly more cigarettes per week than controls ($F(3,142)=4.24$, $p<.01$; *post hoc* $p<.01$) and both occasional and dependent cocaine users consumed more alcohol (grams per week) than MDMA users ($F(3,142)=4.031$, $p<.01$; *post hoc* $p<.01$, $p<.05$) but not in comparison to controls.

Table 1. Demographic data and color vision impairment types, color confusion index (CCI), and total color distance score (TCDS)
(means and standard deviations in parentheses, number of subjects and percent)

	Stimulant-naïve Controls (n=47)	**Occasional MDMA users (n=23)**	**Occasional Cocaine users (n=47)**	**Dependent Cocaine users (n=29)**	***F/Chi²/t***	**df/df$_{err.}$**	***P***	**Effect size**
Age	26.85 (6.95)	23.22 (5.37)	27.21 (7.14)	35.90 (10.86)	13.47[a]	3/142	<0.001	.22[e]
Sex (m, f)	39, 8 (83%, 17%)	22, 1 (96%, 4%)	39, 8 (83%, 17%)	22, 7 (76%, 24%)	3.81[b]	3	0.29	
Years of education	11.55 (1.46)	11.26 (1.74)	10.96 (1.68)	9.50 (1.15)	11.45[a]	3/142	<0.001	.20[e]
Verbal IQ (MWT-B)	107.06 (10.35)	101.43 (11.02)	104.11 (11.14)	103.10 (11.82)	1.62[a]	3/142	0.19	
Smokers/Nonsmokers	35, 12 (75%, 25%)	19, 4 (83%, 17%)	40, 7 (85%, 15%)	27, 2 (93%, 7%)	4.47[b]	3	0.20	
Cocaine Craving (CCQ)	-	-	18.64 (9.29)	19.52 (9.12)	-0.39[d]	69	0.70	
Color vision impairment								
Type I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00[c]	3	1.000	
Type II	1 (2%)	0 (0%)	4 (9%)	3 (10%)	4.51[c]	3	0.211	.18[f]
Type III	4 (9%)	6 (26%)	14 (30%)	10 (35%)	8.99[c]	3	**0.029**	.25[f]
Type IV	0 (0%)	0 (0%)	0 (0%)	1 (3%)	4.06[c]	3	0.255	.17[f]
Total	5 (11%)	6 (26%)	18 (38%)	14 (48%)	14.85[c]	3	**0.002**	.32[f]
CCI	1.09 (0.14)	1.16 (0.16)	1.30 (0.33)	1.40 (0.49)	8.00[a]	3/142	**<0.001**	.14[e]
CCI (range)	1.00 - 1.60	1.00 - 1.64	1.00 - 2.31	1.00 - 3.36				
TCDS	61.67 (7.84)	65.14 (9.05)	73.15 (18.81)	79.09 (27.74)	8.19[a]	3/142	**<0.001**	.15[e]

[a]ANOVA (over all groups), [b]Fisher's Exact Test (over all groups) and [c]Chi^2-Test for frequency data, [d]Independent T-Test (cocaine users only), [e]η^2, [f]Cramer's V,
Type I = red-green, Type II = red-green and blue-yellow, Type III = blue-yellow, Type IV = no clear pattern.

Table 2. Drug use parameters (means and standard deviations in parentheses)

	Stimulant-naïve Controls (n=47)	Occasional MDMA users (n=23)	Occasional Cocaine users (n=47)	Dependent Cocaine users (n=29)
Nicotine				
Cigarettes per week	59.33 (65.30)	74.83 (58.46)	83.18 (60.63)	122.64 (97.85)
Years of use	5.78 (7.39)	4.63 (3.23)	7.93 (6.11)	17.81 (11.16)
Alcohol				
Grams per week	115.68 (137.18)	94.18 (106.74)	173.81 (154.12)	281.98 (382.02)
Cocaine				
Grams per week	-	0.18 (0.55)	1.01 (1.08)	5.46 (6.77)
Years of use	-	0.52 (1.09)	4.58 (3.70)	12.32 (7.39)
Cumulative dose (grams)	-	17.95 (47.50)	330.75 (470.01)	6495.38 (9148.92)
Last use (days)	-	34.00 (17.72) n=5	26.29 (34.33)	18.95 (34.58)
Amphetamine				
Grams per week	-	0.77 (1.27)	0.07 (0.24)	0.00 (0.01)
Years of use	-	2.71 (2.02)	1.20 (2.66)	1.28 (4.18)
Cumulative dose (grams)	-	204.41 (264.83)	43.73 (127.13)	15.75 (61.85)
Last use (days)	-	86.33 (180.70) n=18	172.61 (242.65) n=20	81.63 (81.42) n=4
MDMA				
Pills per week	-	1.65 (2.69)	0.03 (0.12)	0.05 (0.12)
Years of use	-	2.79 (2.01)	1.38 (3.49)	2.57 (5.15)
Cumulative dose (pills)	-	442.36 (469.72)	37.88 (116.45)	235.42 (751.43)
Last use (days)	-	60.95 (157.25)	227.20 (280.00) n=14	72.48 (52.01) n=8
Cannabis				
Grams per week	0.31 (0.73)	3.79 (4.55)	2.62 (5.95)	2.25 (5.66)
Years of use	3.15 (5.03)	3.76 (3.11)	6.19 (5.59)	9.63 (11.45)
Cumulative dose (grams)	791.80 (4001.99)	1015.24 (1307.18)	1601.37 (3680.61)	4260.99 (8694.76)
Last use (days)	29.70 (44.94) n=20	15.60 (26.69) n=15	17.62 (28.99) n=35	19.87 (25.12) n=16
Hallucinogens				
Cumulative dose (times)	4.75 (3.37) n=8	27.25 (40.97) n=16	29.83 (65.48) n=23	16.10 (23.14) n=15
Last use (months)	38.50 (43.25) n=8	6.31 (7.29) n=16	40.07 (47.23) n=23	112.60 (135.42) n=15

Use per week, duration of use, and cumulative dose are averaged within the total group. Last use is averaged only for subjects who used the drug. In this case, sample size is shown.

3.4.2 Qualitative analyses of the LD-15

Qualitative analyses revealed a significant group effect regarding the presence of blue-yellow and total CVI impairment (Table 1, Fig. 1). CVI was more frequent in both, occasional ($\chi^2(1)=9.73$, $p<.01$, $OR=5.21$) and dependent cocaine users ($\chi^2(1)=13.55$, $p<.001$, $OR=7.84$), but not in MDMA users (Fisher's exact $p=.159$), when compared to control subjects (Fig. 1). Occasional and dependent cocaine users did not differ from each other. The most frequent type of CVI was along the blue-yellow axis (type III disorder), where occasional ($\chi^2(1)=6.87$, $p<.01$, $OR=4.56$) and dependent cocaine users ($\chi^2(1)=8.05$, $p<.01$, $OR=5.66$) showed significantly more frequent blue-yellow CVI than controls (Fig. 1). A tendency that also MDMA users exhibited type III disorder more frequently than controls was found (Fisher's exact $p=.07$). Once again, occasional and dependent cocaine users did not significantly differ from one another in the frequency of type III disorders. Although there was no

statistically significant difference between the groups regarding the frequency of type II disorders, occasional and dependent cocaine users showed more frequent red-green colour vision impairment than controls and MDMA users.

Approximately half of the subjects in the MDMA group indicated to also use amphetamine on a regular basis. Interestingly, when the MDMA group was divided in a "low" (less than 150 g amphetamine lifetime, mean±SD=52.8±48.6g, n=12) and "high" amphetamine user group (more than 150 g lifetime, mean±SD=371±307g, n=11), none of the low amphetamine MDMA users suffered from blue-yellow CVI, whereas in the high amphetamine MDMA group 55% showed blue-yellow CVI (Fisher's exact $p<.05$, *Phi*=-.429).

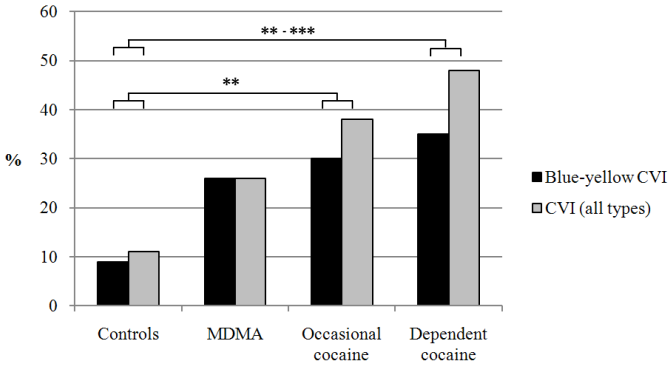

Fig. 1. Frequency of blue-yellow colour vision impairment (CVI) and all types of CVI (%) for stimulant-naive controls, occasional MDMA (commonly known as "ecstasy") users, occasional cocaine users and dependent cocaine users. ** X^2-test: $p<0.01$; *** X^2-test: $p<0.001$.

3.4.3 Quantitative analyses

Quantitative analyses showed that the CCI differed significantly between groups (Table 1), even after controlling for age, sex, alcohol consumption, smoking, and years of education ($F(3,137)=3.72$, $p<.05$, $\eta^2=.08$). *Post hoc* analyses indicated that the CCI of occasional ($p<.05$, $d=.60$) and dependent cocaine users ($p<.05$, $d=.73$) was significantly higher than the CCI of controls. In addition, dependent cocaine users also showed significantly higher CCI scores in comparison to MDMA users ($p<.05$). Occasional and dependent cocaine users did not differ from one another. None of the covariates were significantly related to the CCI.

Confirming the results obtained in the qualitative analysis, high amphetamine MDMA users (CCI=1.25±0.17) showed significantly inferior colour discrimination performance than low amphetamine MDMA users (CCI=1.07±0.08; *post hoc* $p<.01$; $d=1.21$) and controls (CCI=1.09±0.14, $p<.01$; $d=1.10$), while low amphetamine users and controls did not differ (overall all groups, $F(2,67)=6.91$, $p<.01$, $\eta^2=.17$).

To investigate the impact of cannabis use across all four groups, an ANCOVA (covariates see above) with cannabis use (yes/no) and group as fixed-factors was calculated. Neither the factor cannabis use, nor the interaction of group*cannabis use was significant ($F<1.0$), whereas the group

effect remained unchanged ($F(3,133)=2.94$, $p<.05$, $\eta^2=.06$). Given that 19 cocaine users were tested positive for cocaine in urine screening, we investigated the effect of acute cocaine effects on the CCI within the cocaine users. In an ANCOVA (covariates see above) with group (occasional/dependent users) and urine test (positive/negative) as fixed-factors both main effects were not significant ($F<.20$). However, a disordinal group*urine test interaction was found ($F(1,67)=4.12$, $p<.05$, $\eta^2=.06$), indicating that positive tested occasional cocaine users exhibited more pronounced CVI than negative tested occasional users, whereas positive tested dependent users displayed less CVI than negative tested dependent users (Fig. 2).

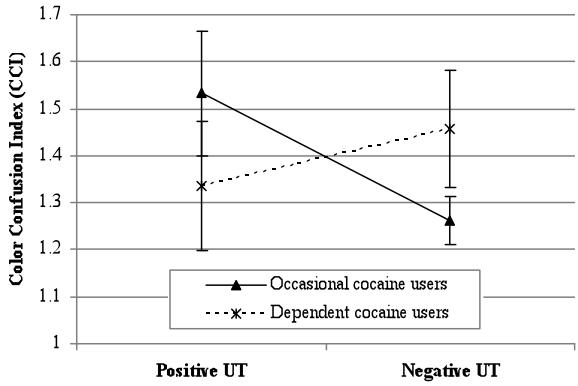

Fig. 2. Significant interaction between cocaine group (47 occasional vs. 29 dependent users) and urine toxicology (UT; 19 positive vs. 57 negative tests) ($F_{1,67}=4.12$, $p<0.05$). Means and SEM are shown.

3.4.4 Sensitivity and specificity of the LD-15

Sensitivity calculations indicated that the LD-15 correctly identified CVI (all types) in cocaine users in 42% of the cases. Specificity was 84%, indicating a false-positive rate of 16% for the controls and MDMA users. The positive predictive value (PPV) or in other words the probability that an individual actually exhibits CVI when a positive test result was observed was 74%. The negative predictive value (NPV) or the probability that an individual without CVI really is free from the condition was 57%. Sensitivity for blue-yellow CVI was 32%, specificity 91%, PPV 71%, and NPV 54%.

3.4.5 Verbal learning and declarative memory performance

To investigate the association of CVI and memory performance, cocaine users with diagnosed CVI (Coc_{CVI}), cocaine users without CVI ($Coc_{non\text{-}CVI}$), and controls without CVI were compared (Table 3). ANCOVA analyses were corrected for age and years of education. Importantly, Coc_{CVI} and $Coc_{non\text{-}CVI}$ did not significantly differ regarding cocaine use parameters. In general, cocaine users showed worse performance in the working memory span, recall after interference, and delayed recall, when compared to controls. Moreover, Coc_{CVI} showed inferior performance than $Coc_{non\text{-}CVI}$ in the supraspan,

learning, recall consistency, delayed recall, and recognition of list A and B. Controls differed from Coc_{CVI} in all memory parameters.

Table 3. Verbal learning/memory performance of controls without color vision impairment (CVI), and cocaine users with and without CVI (means adjusted for age and years of education, and standard deviations in parentheses)

RAVLT parameters	Controls without CVI (n=42)	Cocaine users without CVI (n=44)	Cocaine users with CVI (n=32)	*F*	$df/df_{err.}$	*P*	*Sidak post hoc* group comparisons (*P* < .05)
Working memory span (*trial 1*)	9.58 (1.95)	8.67 (1.86)	8.13 (1.91)	4.91	2/113	<.01	Cont. > $Coc_{non\text{-}CVI}$ > Coc_{CVI}
Learning performance (*trials Σ1-5*)	63.42 (7.45)	60.21 (7.08)	55.38 (7.27)	10.12	2/113	<.001	Cont., $Coc_{non\text{-}CVI}$ > Coc_{CVI}
Recall consistency, trials 1-5 (*in percent*)	92.67 (9.75)	90.04 (9.27)	84.71 (9.51)	5.95	2/113	<.01	Cont., $Coc_{non\text{-}CVI}$ > Coc_{CVI}
Interference trial (*list B*)	8.97 (2.42)	8.29 (2.30)	7.36 (2.36)	3.88	2/113	<.01	Cont. > Coc_{CVI}
Recall after interference (*trial 6*)	13.44 (2.37)	12.14 (2.26)	11.25 (2.31)	7.54	2/113	<.01	Cont. > $Coc_{non\text{-}CVI}$, Coc_{CVI}
Delayed recall (*trial 7 after 30 min*)	13.55 (2.11)	12.53 (2.00)	11.27 (2.06)	10.03	2/113	<.001	Cont. > $Coc_{non\text{-}CVI}$ > Coc_{CVI}
Loss after consolidation (*trial 5 minus 7*)	0.65 (1.47)	1.13 (1.40)	1.65 (1.44)	3.98	2/113	<.01	Cont. > Coc_{CVI}
Adjusted recognition list A (*p(A) list A*)	0.90 (0.11)	0.87 (0.11)	0.82 (0.11)	4.98	2/113	<.01	Cont., $Coc_{non\text{-}CVI}$ > Coc_{CVI}
Adjusted recognition list B (*p(A) list B*)	0.82 (0.13)	0.77 (0.12)	0.70 (0.12)	6.73	2/113	<.01	Cont., $Coc_{non\text{-}CVI}$ > Coc_{CVI}

CVI=Color vision impairment, Cont.=Controls without CVI, Coc_{CVI}=cocaine users with CVI, $Coc_{non\text{-}CVI}$=cocaine users without CVI.

In a second step we investigated whether RAVLT performance scores were correlated with CCI sores and cocaine use (Table 4). Control subjects have been excluded from this analysis in order to prevent inflation of existing associations and MDMA users because previous findings have shown that MDMA users exhibit marked verbal declarative memory deficits (Quednow et al., 2006). After controlling for age, the CCI correlated negatively with the supraspan, learning performance, delayed recall, adjusted recognition for list A, and positively with loss after consolidation, reflecting that more pronounced CVI was associated with worse memory performance. When corrected for age, cocaine use, particularly lifetime use, was correlated with several memory parameters (Table 4), indicating worse memory performance with increasing cocaine use. Last cocaine use in days was not significantly related to any of the RAVLT variables (data not shown).

Table 4. Correlations between memory performance, color confusion index (CCI), and cocaine use parameters in cocaine users (partial correlation r and p-value in parentheses)

	CCI	Cocaine (g / week)	Cocaine (years of use)	Cocaine (lifetime in g)	Cocaine (max. in g / day)
Supraspan	**-.27 (.02)**	-.22 (.06)		**-.36 (.001)**	**-.39 (.004)**
Learning performance (trials ∑1-5)	**-.33 (.004)**	**-.26 (.02)**	**-.25 (.03)**	**-.38 (.001)**	**-.33 (.006)**
Recall consistency, trials 1-5 (in percent)		-.20 (.09)	-.23 (.06)	**-.30 (.01)**	-.22 (.06)
Interference trial (list B)	-.19 (.10)			-.21 (.07)	-.20 (.09)
Recall after interference (trial 6)	-.19 (.10)	**-.34 (.003)**	**-.29 (.01)**	**-.33 (.004)**	**-.27 (.03)**
Delayed recall (trial 7 after 30 min)	**-.27 (.02)**	-.21 (.07)	-.20 (.09)	**-.31 (.008)**	
Loss after consolidation (trial 5 minus 7)	**.24 (.04)**				
Adjusted recognition list A (p(A) list A)	**-.26 (.02)**		**-.30 (.008)**	**-.33 (.004)**	**-.28 (.02)**
Adjusted recognition list B (p(A) list B)	-.22 (.06)	-.22 (.06)	-.22 (.06)	**-.39 (.001)**	**-.36 (.002)**

Correlations are adjusted for age. Correlations with a *p*-level below 10% are shown, while significant correlations ($p<.05$) are in bold.

3.4.6 Association between CVI, substance use and demographic data

In the total sample, CCI sores were correlated with age (r=.21, p<.05), years of education (r=-.19, p<.05), cigarettes smoked per week (r=.17, p<.05), and years of smoking (r=.22, p<.01), indicating worse colour discrimination with increasing age, lower education, and higher nicotine use. Alcohol and cannabis use, gender, and verbal IQ were not associated with the CCI.

For the correlations between illegal drug use and the CCI (except for cannabis), control subjects were excluded to prevent inflating existing correlations. Alcohol, cannabis, amphetamine, and MDMA use were not associated with the CCI. Only cocaine use in years (r=.25, p<.05, n=99) and cocaine lifetime use in grams (r=.25, p<.05, n=99) were positively correlated with the CCI. The effects remained after adjusting for age (both r=.20, p<.05). A binary logistic regression analysis (stepwise forward) examining the association of age, verbal IQ, years of education, cigarettes per week, alcohol use (g/week), lifetime use of cannabis, amphetamine, cocaine (in grams), and MDMA (in pills), with CVI indicated that the presence of dyschromatopsia was only related to cocaine lifetime use (β=0.36, p<.05), whereas no significant associations were found with any other variables. Cocaine craving (CCQ) was not significantly correlated with the CCI. Furthermore, Coc_{CVI} and $Coc_{non\text{-}CVI}$ did not significantly differ in their CCQ scores (data not shown).

3.5 Discussion

Occasional and dependent cocaine users both showed more frequent and more intense CVI, predominantly in the blue-yellow spectrum, in comparison to psychostimulant-naïve controls. CVI in dependent cocaine users was more pronounced than in occasional cocaine users as indicated by the larger effect sizes and the higher prevalence of CVI in the qualitative data. However, it is noteworthy that CVI was already highly frequent in occasional cocaine users. MDMA users with low exposure to dopaminergic stimulants did not show changes in colour vision but MDMA users who had often used amphetamine showed similar blue-yellow CVI as cocaine users. Higher CCI was related to higher cumulative lifetime cocaine use and longer duration of use. Moreover, the presence of CVI was clearly associated with diminished verbal declarative memory performance in cocaine users, possibly reflecting a potential relationship between retinal and cerebral dopaminergic alterations. Overall, these results support the notion that CVI, particularly blue-yellow CVI, is specific for drugs mainly altering the dopamine system such as cocaine and amphetamine.

The present findings are largely consistent with previous studies providing evidence that blue-yellow CVI prevails in withdrawn cocaine-dependent patients (Desai et al., 1997; Roy et al., 1996, 1997*a*, *b*, 2003). However, our report elaborated on this finding by showing that already occasional cocaine and amphetamine use was associated with blue-yellow CVI and that a different pattern

applies for occasional stimulant use regarding abstinence. The authors of prior studies proposed that the CVI was due to the effect of cocaine on dopaminergic retinal neurotransmission leading to a hypodopaminergic state and not owing to the effect of cocaine on ocular blood vessels, as a careful examination indicated no retinal lesions. Compared to Desai et al. (1997) who reported that 48% of the dependent cocaine users exhibited blue-yellow CVI, a lower prevalence of 35% for dependent and 30% for occasional cocaine users was found in the present sample. However, this may be due to slightly different error classifications between both studies as we found that 48% of the dependent and in 38% of the occasional cocaine users showed CVI when all types of CVI were considered. Furthermore, in our study 19 of 76 users tested positive for cocaine in urine toxicology and may not have been suffering from an acute hypodopaminergic state. In accordance to Desai et al. (1997), no significant correlation between CVI and days of cocaine abstinence was found in the present results. Moreover, Roy et al. (1996, 1997*a*) found that cocaine-dependent patients with an ERG blue cone b-wave amplitude less than 0.5 mV reported stronger cocaine craving, which is in line with the finding that reduced D_2 receptor binding in the dorsal striatum during a cocaine-cue condition was linked to self-reports of cocaine craving (Volkow et al., 2006). In contrast, cocaine users with CVI in the present sample did not report stronger cocaine craving than cocaine users without CVI. Given that Roy et al. (1996, 1997*a*, *b*) had used the 45-item version of the CCQ, differences in the magnitude of the reported craving scores cannot be directly compared. Nevertheless, cocaine users from the present study may have experienced less craving as several users had tested positive for cocaine in the urine analysis.

Earlier studies have not examined the relationship between CVI and drug use patterns in detail. In the present report, CVI was correlated with lifetime quantity and duration of cocaine use, supporting the view that CVI might be cocaine-induced. However, the cross-sectional design of the study does not allow a final conclusion regarding causality. Even though dependent cocaine users were more strongly impaired in colour vision discrimination than occasional cocaine users, the relatively small difference between the two groups raises the question whether a hypodopaminergic state could be a pre-morbid trait that functions as a risk factor or possibly that CVI may be due to an interaction of predisposition and substance-induced effects. Although abstinence period since last cocaine use was not directly related to LD-15 performance, it is noteworthy that occasional cocaine users showed better colour discrimination when the urine toxicology was tested negative for recent cocaine use, while dependent cocaine users performed better when they were tested positive. A potential explanation could be that the hypodopaminergic state was less severe in occasional cocaine users, wherefore acute dopamine release had no beneficial effect on their colour discrimination ability. Furthermore, neither alcohol nor any of the other psychoactive drugs were significantly related to CVI in cocaine users. Finally, it was not possible to identify a threshold value as to after which cocaine

doses CVI occurs. However, for both occasional and dependent cocaine users, duration and quantity of cocaine use were most strongly related to CVI.

MDMA users who had co-used amphetamine showed blue-yellow CVI, while MDMA users who had used little amphetamine did not exhibit blue-yellow CVI. The correlation between CVI and amphetamine use was not significant, but only few subjects reported amphetamine use and thus the power to detect a dose-response relationships was low. Although some animal studies have yielded evidence that MDMA, at least acutely and in a much less potent manner than amphetamine-like derivates (Partilla et al., 2006), also increases synaptic dopamine levels and dopamine efflux (Baumann et al., 2007; Yamamoto and Bankson, 2005), the majority of the studies only reported selective neurotoxicity for 5-HT-containing neurons (for review, see Capela et al., 2009). It is currently controversial if cocaine use can also be neurotoxic for dopaminergic neurons; however, recent human post-mortem studies support this notion (Little et al., 2009; Okvist et al., 2011).

We have demonstrated that CVI was associated with working memory span, encoding, and retrieval deficits in cocaine users. In line, with the present results, several measures of verbal memory, attentional performance, and executive function have consistently revealed deficits in withdrawn cocaine users (Fernandez-Serrano et al., 2009; Goldstein et al., 2004; Kelley et al., 2005; Woicik et al., 2009). Many cognitive processes, working memory and executive functions in particular, are mediated by the PFC, which is strongly dependent on intact dopamine signalling (Braskie et al., 2008; Nieoullon, 2002; Vernaleken et al., 2007). Accordingly, cocaine users with CVI displayed worse performance in memory parameters previously associated with the PFC such as the working memory span and the recall consistency (Quednow et al., 2006). Given that cocaine use may exert its influence on both cognition and colour vision, a possible link between alterations in the frontostriatal and retinal dopamine system is self-evident. Prior studies have indeed provided evidence pointing in this direction. As mentioned above, decreased blue cone b-wave ERG amplitudes in dependent cocaine users were associated with lower concentrations of CSF HVA (Roy et al., 2003) and reduced striatal D_2 receptor availability was related to lower metabolic activity in the medial PFC and the anterior cingulate gyrus persisting three to five months after detoxification (Volkow and Li, 2004; Volkow et al., 1988, 1991, 1993). However, the specific mechanism of how dopamine mediates colour vision discrimination remains elusive and it is unclear how stimulant use precisely affects retinal dopamine transmission. Naturally, the question arises to what extent alterations in the frontostriatal and retinal dopamine system may be linked. Combining cerebral molecular imaging with ophthalmological/electrophysiological methods could greatly contribute to a better understanding of the exact mechanism by which blue-yellow CVI and cognitive dysfunction occur. In some studies, it was postulated that short-wavelength-sensitive cones (blue cones) may generally be more vulnerable to toxic noxa and aging than medium- and long-wavelength-sensitive cones, possibly due to their relative scarcity and anatomical distribution (Djamgoz et al., 1997; Hart, 1987; Masson et al., 1993;

Witkovsky, 2004). Moreover, mammalian studies reported that certain cell types in the retina have selective blue-cone input that is mediated by dopamine transmission (Djamgoz et al., 1997).

Eventually, the usefulness of the LD-15 as a marker for dopaminergic and cognitive alterations shall be addressed. The present results imply that although the specificity of the LD-15 was good its sensitivity is not high enough for diagnostic and predictive purposes regarding blue-yellow CVI in cocaine users. Roy et al. (1997*a*, *b*, 2003) have previously suggested that ERG blue cone b-wave amplitudes may serve as a neurobiological marker related to central dopamine function in cocaine-dependent patients. It would be of interest to further investigate the correspondence between ERG blue cone amplitudes with the LD-15 and cognitive deficits.

Some limitations inherent to the investigation of CVI were present in this study. Colour vision is influenced by several factors besides stimulant use, such as age, alcohol and nicotine use. However, occasional cocaine users, MDMA users and controls were well matched and potentially confounding variables were controlled in the analyses. The sensitivity of the LD-15 is limited and blue-yellow CVI also occurred in controls, although to a much lower extent. Furthermore, the possibility that some cocaine users may have suffered minor ocular blood vessel bursts cannot be ruled out since participants did not undergo an ophthalmological examination. Nevertheless, results from prior studies controlling for blood vessel damage are consistent with the findings of the current study (Desai et al., 1997; Roy et al., 1996). Moreover, in addition to alterations in dopaminergic transmission in the retina, sub-cortical and cortical processing may also be affected and contribute to decreased colour discrimination ability (Conway et al., 2010). Finally, the history of drug consumption was assessed only by means of self-reports, precluding to calculate lifetime drug use objectively (Curran, 2000).

3.5.1 Conclusion

In conclusion, this study provided evidence that drug users consuming stimulants that mainly alter the dopaminergic neurotransmitter system exhibit more frequent blue-yellow CVI and already stimulant users with an occasional cocaine use pattern are affected to a similar extent as chronic users. Furthermore, CVI was found to be associated with decreased performance in verbal learning and memory in occasional and dependent cocaine users, implying that retinal and cerebral dopaminergic alterations could be linked to a certain degree.

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Conflict of interest

Experimental design, data acquisition, statistical analyses, and interpretation of the results were conducted without input from any pharmaceutical company. All authors report no biomedical financial interests or potential conflicts of interest with respect to this study.

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NICOTINE BUT NOT COCAINE USE IS ASSOCIATED WITH DECREASED CEREBRAL METABOTROPIC GLUTAMATE RECEPTOR 5 DENSITY IN HUMANS

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Personal contribution

LMH collected and analyzed the data, interpreted the data, and wrote the manuscript. BBQ conceived and designed the study, and revised the first draft of the manuscript. VT helped with the PET analyses. AJ, SMA, and AB contributed analytic tools. KHP, MV, and ES contributed to data acquisition and/or revised the first draft of the manuscript. MRB contributed hair toxicology analyses.

4.1 Abstract

Background: Long-lasting neuroadaptations in the glutamatergic cortico-striatal circuitry have been suggested to be responsible for the persisting nature of drug addiction. In particular, animal models have linked the metabotropic glutamate receptor 5 (mGluR5) to drug-seeking behavior and extinction learning. Accordingly, blocking mGluR5s attenuated self-administration of cocaine and other addictive drugs in rats. How these animal findings extend to humans remains unclear. Therefore, we investigated if human cocaine users exhibit altered mGluR5 availability compared to drug-naïve control subjects.

Methods: Eighteen male controls (12 smokers) and 18 male cocaine users (13 smokers) underwent positron emission tomography with ^{11}C-ABP688 to quantify mGluR5 availability in addiction-related brain areas. Drug use was assessed by self-report and quantitative hair toxicology. Twelve volumes-of-interest implicated in addiction were defined.

Results: Cocaine users and controls did not significantly differ in mGluR5 availability. In contrast, smokers (n=24) showed a significant reduction in mGluR5 density throughout the brain (mean 20%) compared to non-smokers (n=11). In terms of effect sizes, reductions of mGluR5 availability were most pronounced in the caudate nucleus (d=1.50, 21%), insula (d=1.47, 20%), and putamen (d=1.46, 18%). Duration of nicotine abstinence was positively related to mGluR5 density in all brain regions-of-interest, indicating that mGluR5 expression was particularly decreased in individuals who had smoked very recently.

Conclusions: Nicotine use was associated with decreased mGluR5 availability in both cocaine users and controls, while cocaine use was not linked to detectable mGluR5 alterations. These findings have important implications regarding the development of novel pharmacotherapies aimed at facilitating smoking cessation.

4.2 Introduction

Cocaine addiction continues to be a major public health concern resulting in high societal and economic costs (Degenhardt and Hall, 2012; Olesen et al., 2012). Notably, cocaine is the second most used illicit drug with 13 to 20 million users worldwide, corresponding to annual global cocaine use levels of 0.3-0.4% and annual prevalence rates of 1.6% in North America, 1.3% in Central Europe, and 1.5-1.9% in Oceania (UNODC, 2012). The high prevalence rates of cocaine use and its harmful effects also become evident through the high treatment demand related to psychostimulants. Accordingly, 17% of all patients who entered drug treatment programs in the USA (SAMHSA, 2010), and 23% of the drug-seeking patients in Europe did so for the treatment of stimulant addiction (EMCDDA, 2011). Despite the high treatment demand, effective pharmacotherapies for cocaine addiction are currently lacking (O'Brien, 2005; Olive et al., 2012).

A core feature of cocaine addiction is ongoing compulsive drug use despite the encounter of potentially adverse physical, psychological, or social consequences resulting in frequent relapses (Association, 2000; Hyman and Malenka, 2001; Koob, 2009). While acutely reinforcing effects of addictive drugs are mainly dependent on the mesocorticolimbic dopamine system (Volkow et al., 2012), accumulating preclinical research has provided evidence that longer-lasting neuroadaptations in glutamatergic corticolimbic circuitries may be responsible for the persisting nature of cocaine addiction (Everitt and Robbins, 2005; Kalivas, 2009; Nestler, 2002; Thomas et al., 2008; Wolf et al., 2004). In particular, glutamatergic projections from the prefrontal cortex (PFC) to the nucleus accumbens (NAcc) appear to be relevant for drug reinstatement and drug-seeking behavior in animals and may also play an important role in craving and relapse-related behaviors in psychostimulant-addicted humans (Kalivas, 2009; Kalivas and Volkow, 2005; McFarland et al., 2003). Chronic self-administration in rats resulted in lower basal extracellular, non-synaptic glutamate levels in the NAcc, which in turn has been associated with down-regulation of group I and II metabotropic glutamate receptor (mGluR) expression and function in rats (Ary and Szumlinski, 2007; Ben-Shahar et al., 2009; Fourgeaud et al., 2004; Ghasemzadeh et al., 2009; Hao et al., 2010; Swanson et al., 2001), resulting in altered synaptic plasticity. Such impaired plasticity has been suggested to contribute to the relative inability to form adaptive behaviors that help inhibiting relapse in drug users (Kalivas, 2009).

The mGluR of type 5 (mGluR5), belonging to group I mGluRs, has gained growing attention in addiction research due to its high expression in corticolimbic regions implicated in drug addiction including the mPFC, OFC, cingulate, striatum, amygdala, and hippocampus (Abe et al., 1992) and involvement in drug-seeking behavior and extinction learning in animals withdrawn after cocaine self-administration (for reviews Bellone and Mameli, 2012; Duncan and Lawrence, 2012; Kenny and Markou, 2004; Olive, 2010). In fact, mGluR5 null mutant mice did not self-administer cocaine or

exhibit increased locomotor activity after cocaine treatment (Chiamulera et al., 2001) and self-administration and reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin was attenuated by mGluR5 antagonists (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Kenny et al., 2005; Kumaresan et al., 2009; Lee et al., 2005; Martin-Fardon et al., 2009; Paterson and Markou, 2005; Paterson et al., 2003; Platt et al., 2008; van der Kam et al., 2007). Therefore, much effort is being undertaken to develop pharmacological compounds with the ability to block mGluR5s, which might be one efficacious way to treat stimulant-addiction.

How preclinical findings regarding changes in mGluR5 expression extend to human cocaine users has not been investigated to date, mainly due to lack of suitable radioligands. New methodological advances have made it possible to quantify mGluR5 availability in humans with the mGluR5-selective positron emission tomography (PET) radioligand ^{11}C-ABP688 (3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-enone-O-carbon-11-methyl-oxime (Ametamey et al., 2006; Ametamey et al., 2007). Recently, a preliminary PET study using ^{11}C-ABP688 demonstrated that smokers have globally decreased mGluR5 density in the brain (Akkus et al., 2013). However, it is not clear if mGluR5 alterations are specific for nicotine addiction or a common phenomenon in drug addiction. Therefore, the aim of the present study was to quantify mGluR5 availability in brain regions with high mGluR5 expression and implicated in addiction employing ^{11}C-ABP688 PET in cocaine users and drug-naïve controls. Moreover, a second aim was to investigate the potential impact of smoking on mGluR5 availability in cocaine users. We expected, that both cocaine and nicotine alter mGluR5 availability.

4.3 Methods and materials

4.3.1 Participants

The study sample consisted of 18 male drug-naïve healthy control subjects (HC) and 18 male cocaine users (CU) who also took part in the longitudinal Zurich Cocaine Cognition Study (ZuCo^{2}St) (Hulka et al., 2012; Preller et al., 2012; Vonmoos et al., 2012). For the ZuCo^{2}St, participants were recruited via advertisements in local newspapers, different drug prevention and treatment centers, psychiatric hospitals, internet platforms, and word-of-mouth communication (for details please see: Preller et al., 2012). Inclusion criteria for CU were 1) diagnoses of cocaine abuse or dependence according to DSM-IV as assessed by the Structured Clinical Interview (Wittchen et al., 1997), 2) abstinence duration of cocaine use <6 months, 3) age between 20 to 50 years, 4) proficiency in German language, 5) no polytoxic drug use pattern according to DSM-IV and no use of opioids or prescription drugs affecting the CNS, 6) no current or previous Axis I DSM-IV psychiatric disorder (other than cocaine abuse/dependence, alcohol abuse in cocaine users, and a former depressive

episode), 7) no neurological disorder or head injury, 8) no family history of a severe DSM-IV psychiatric disorder such as schizophrenia, bipolar disorder or obsessive-compulsive disorder, and 9) no metallic particles in the body. The same inclusion criteria applied to HC except for 1) and 2). Additionally, CU were excluded when they displayed any psychiatric disorder according to DSM-IV, and any regular illegal drug use (lifetime use <15 occasions) with exception of cannabis. Participants were instructed to abstain from illegal drugs for a minimum of three days and from alcohol for at least 24 hours. Urine samples were collected to control for recent drug use. To objectively characterize drug use over the past six months, 6cm hair samples were collected and analyzed with liquid chromatography-mass spectrometry (LC-/MS, for details see **Supplement**). Participants received financial compensation. This study was approved by the Ethics Committee of the Canton Zurich and the Swiss Federal Office of Public Health. After receiving a written and oral description of the aim of this study, all participants gave written informed-consent before inclusion.

4.3.2 Clinical interviews and questionnaires

The *Structured Clinical Interview for DSM-IV Disorders* was carried out by a trained psychologist (Wittchen et al., 1997). Drug use was assessed by means of the *Interview for Psychotropic Drug Consumption*, which has been described in detail elsewhere (Quednow et al., 2004). Symptoms of depression were measured by means of the *Beck Depression Inventory* (BDI) (Beck et al., 1961). The *Symptom Checklist-90-R* (SCL-90-R) was used as a screening measure of general psychiatric symptoms (Franke, 1995). The brief version of the *Cocaine Craving Questionnaire* (CCQ) was used to assess current cocaine craving (Sussner et al., 2006; Tiffany et al., 1993). The *Fagerström Test for Nicotine Dependence* (FTND) was used to determine severity of nicotine dependence (Heatherton et al., 1991).

4.3.3 Image acquisition and data analyses

Magnetic Resonance Image Acquisition. T1-weighted magnetic resonance (MR) imaging was acquired for each participant on a Philipps Achieva 3T whole-body scanner equipped with an 8-channel head array (Phillips Healthcare, Best, The Netherlands) to rule out structural abnormalities and for the preprocessing of PET images.

PET Acquisition. The synthesis of ^{11}C-ABP688 has been described in detail elsewhere (Ametamey et al., 2006; Ametamey et al., 2007). PET image acquisition was conducted in 3-D mode in the whole-body scanner DSTX PET/computed tomography scanner (B. Braun Medical, Sempach, Switzerland) at the University Hospital Zurich (Division of Nuclear Medicine). Prior to positioning

the participants in the scanner, a catheter was placed in the left antecubital vein for tracer injection. Before the PET acquisition, a low-dose CT was conducted for attenuation correction. In order to avoid arterial blood sampling, which is inconvenient for participants, we relied on a previously evaluated equilibrium paradigm that can be achieved with a bolus-infusion protocol (Burger et al., 2010; Treyer et al., 2007). The equilibrium paradigm is based on the premise that a steady-state between tracer concentration in tissue and blood can be obtained (Blasberg et al., 1989; Carson et al., 1993). ^{11}C-ABP688 was administered according to a bolus-infusion protocol (Burger et al., 2010; Carson et al., 1993), where half of the tracer was administered as a bolus over 2 minutes and the other half was infused over 58 minutes using an infusion pump (Perfusor FM, Braun Medical). Groups did not differ regarding injected activity [HC: 604±29 MBq, CU: 600±24 MBq, $t(33)=-0.42$, $p=.68$ (two-tailed)]. At tracer injection, a series of 20 scans was recorded over a total duration of 60min with the first 10 frames lasting 60sec and the remaining 10 scans each lasting 300sec. Applying filtered back-projection, transaxial images were reconstructed to a 128x128 matrix with 47 transaxial slices of 2.3x2.3x3.2mm voxel size.

PET Image Processing and Quantification. Image processing and quantification steps were performed with the PMOD software, version 3.307 (PMOD Technologies, Zurich, Switzerland, www.pmod.com). Initially, the average of frames 2 to 16 was realigned to the average of frames 17 to 19 (rigid matching), thereby correcting for within-subject motion. Subsequently, the averaged frames 17 to 19 were spatially normalized to the Montreal Neurological Institute (MNI) template brain. Twelve volumes of interest (VOIs) and a cerebellar reference VOI were generated based on the standard VOIs of the MNI template brain (Tzourio-Mazoyer et al., 2002) comprising the following brain regions: anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), medial prefrontal cortex (MPFC), orbitofrontal cortex (OFC), ventrolateral prefrontal cortex (VLPFC), nucleus caudatus, putamen, insula, amygdala, parahippocampal gyrus, hippocampus, and thalamus.

To verify that steady-state of receptor binding was reached after 45min. (frames 17 to 19), time-activity curves (TACs) were generated for high receptor density regions (ACC, putamen), medium receptor density region (thalamus), and low receptor density regions (cerebellum) in both hemispheres for all participants. Steady state of ^{11}C-ABP688 uptake 45min after tracer injection was reached in all participants except for one control subject, who was therefore excluded from the subsequent statistical analyses. Normalized values of distribution ($V_{norm}=C_{T[VOI]}/C_{T[Cer]}$) of ^{11}C-ABP688 uptake constituted the quantitative PET outcome measure and was derived by dividing the average radioactivity concentration between 45 and 55 minutes (frames 17 to 19) of each VOI ($C_{T[VOI]}$) with the commensurate cerebellar radioactivity concentration ($C_{T[Cer]}$). V_{norm} is equivalent to $BP_{ND}+1=((V_T-V_{ND})/V_{ND})+1)$ (Innis et al., 2007). mGluR5 has been successfully quantified with this approach in previous studies (Burger et al., 2010; Deschwanden et al., 2011; Hefti et al., 2012) and a preclinical

study showed that the cerebellum is relatively devoid of mGluR5 and thus suitable as a reference region (Elmenhorst et al., 2010).

4.3.4 Statistical analysis

Statistical analyses were performed with the PASW 19.0 software (SPSS Inc.). Independent t-tests and frequency analyses (Pearson's Chi^2 test) were conducted to compare groups among demographic and clinical questionnaire data. PET data were analyzed by means of a mixed-effect analysis of variance (ANOVA) including the between-subject factors *group* (HC, CU) and *smoking status* (non-smokers, smokers), the within-subject factors *brain region* (12 VOIs) and *hemisphere* (left, right), and their interactions. Two-tailed independent t-tests were conducted to identify differences of ^{11}C-ABP688 uptake in the selected VOIs between groups. To account for multiple testing, the Bonferroni correction was applied, resulting in a significance level of $p<.0042$ ($p=.05/12$). Potential associations of drug use parameters and mGluR5 availability were examined with correlation analyses (Pearson's product-moment). Effect sizes (Cohen's *d*) were calculated with G*Power 3.1 (Faul et al., 2007). As the assumption of parametric distribution was not met by certain variables, the drug use parameters weekly alcohol, nicotine, cannabis and cocaine consumption (in grams/cigarettes), last alcohol, nicotine, cannabis and cocaine use (hours), cumulative cannabis and cocaine dose (in grams), and concentrations of cocaine, benzoylecgonine, ethylcocaine and norcocaine in hair samples (pg/mg) were log-transformed (log10) and the constant 1 was added because the data contained 0 values.

4.4 Results

4.4.1 Demographic information and drug use patterns

HC and CU were well-matched for age, smoking status, years of education, and did not differ with regard to BDI, SCL-90, and FTND scores **(Table 1)**. Nine (50%) of the CU met the DSM-IV criteria for current cocaine dependence, while the remaining 9 (50%) CU met cocaine abuse criteria. Except for one CU who reported to smoke crack cocaine, all other CU indicated to use cocaine nasally. None of the participants met the criteria for alcohol dependence. Drug use patterns are shown in **Table 2**. Regarding substances that were used in both groups no significant differences emerged in nicotine use. However, CU reported higher weekly alcohol consumption [$t(33)=-2.84$, $p<.05$], higher cumulative doses of cannabis [$t(33)=-2.17$, $p<.05$], and by trend higher weekly cannabis use [$t(33)=-1.96$, $p=.07$].

Table 1. Demographic data (means and standard deviations, number of subjects and percent)

	Controls (n=17)	**Cocaine users (n=18)**	**Value[a]**	***p*[a]**	***df***
Age	36.24 (±8.34)	36.17 (±7.64)	0.03	0.98	33
Body Mass Index (kg/m^2)	23.72 (±2.77)	25.09 (±3.51)	-1.28	0.21	33
Years of education	10.53 (±1.94)	10.50 (±1.89)	0.05	0.96	33
Beck Depression Index (BDI)	5.59 (±6.54)	5.89 (±3.91)	-0.17	0.87	33
SCL-90-R Global Severity Index (GSI)	0.31 (±0.42)	0.38 (±0.24)	-0.61	0.55	33
SCL-90-R Positive Symptom Distress Index (PSDI)	1.19 (±0.28)	1.28 (±0.25)	-1.00	0.33	33
SCL-90-R Positive Symptom Total (PST)	20.18 (±22.35)	25.72 (±14.09)	-0.88	0.38	33
Cocaine Craving Questionnaire (CCQ) (sum)	-	20.06 (±6.03)	-	-	-
Smoking Status (yes/no)	11, 6 (65, 35%)	13, 5 (72, 28%)	0.23	0.63	1
Fagerström (sum)	2.55 (±2.73)	4.85 (±3.21)	-1.87	0.08	22

[a]Independent T-test, [b]Chi^2-test for frequency data.

CU had a mean weekly cocaine consumption of 1.5g, while they reported relatively very little co-use of amphetamine and MDMA. Hair toxicology analyses capturing the past six months confirmed that cocaine was the primary drug of choice in all CU. Methamphetamine and opiates were not detected, while mean amphetamine values were far below the threshold of reliable detection >200 pg/mg (Cooper et al., 2012). It is noteworthy that 44% of the CU tested positive for cocaine in the urine toxicology analysis. Therefore, additional analyses were carried out to investigate post-acute effects of cocaine use.

Table 2. Drug use patterns (means and standard deviations)

	Controls (n=17)	**Cocaine users (n=18)**
Alcohol		
Grams per week	88.10 (±62.90)	262.00 (±251.48)*
Years of use	17.41 (±8.83)	17.58 (±6.46)
Nicotine		
Cigarettes per week	54.15 (±63.08)	85.19 (±73.74)
Years of use	11.32 (±11.09)	12.06 (±9.18)
Last consumption (hours)	14.47 (±22.36) n=11	123.86 (±320.35) n=13
Cannabis		
Grams per week	0.16 (±0.43)	1.07 (±1.92)†
Years of use	4.79 (±7.59)	7.83 (±8.67)
Cumulative dose (grams)	177.23 (±290.81)	1563.85 (±2689.90)*
Last consumption (days)	50.76 (±74.39) n=5	3.81 (±2.14) n=8
Urine toxicology (pos./neg.)	2, 15 (12, 88%)	6, 12 (33, 67%)
Cocaine		
Times per week	0.00 (±0.00)	1.28 (±1.39)
Grams per week	0.00 (±0.00)	1.46 (±1.36)
Years of use	0.00 (±0.00)	10.14 (±5.55)
Maximum dose (grams/day)	-	3.94 (±2.89)
Cumulative dose (grams)	0.00 (±0.00)	1056.56 (±977.50)
Last consumption (days)	-	7.60 (±6.84)
Cocaine in hair (pg/mg)	0.00 (±0.00)	18915 (±19665)
Benzoylecgonine in hair (pg/mg)	0.00 (±0.00)	3460 (±3575)
Ethylcocaine in hair (pg/mg)	0.00 (±0.00)	1490 (±2710)
Norcocaine in hair (pg/mg)	0.00 (±0.00)	395 (±500)
Urine toxicology (pos./neg.)	0, 100 (0, 100%)	8, 10 (44, 56%)
Amphetamine		
Grams per week	0.00 (±0.00)	0.0043 (±0.01)
Years of use	0.00 (±0.00)	0.64 (±1.30)
Cumulative dose (grams)	0.00 (±0.00)	10.77 (±25.86)
Last consumption (days)	-	76.00 (±40.22) n=3
Amphetamine in hair (pg/mg)	0.00 (±0.00)	12 (±30) n=3
Methamphetamine in hair (pg/mg)	0.00 (±0.00)	0.00 (±0.00)
MDMA		
Pills per week	0.00 (±0.00)	0.03 (±0.06)
Years of use	0.00 (±0.00)	3.58 (±4.94)
Cumulative dose (pills)	0.00 (±0.00)	24.88 (±63.74)
Last consumption (days)	-	70.63 (±47.67) n=7
MDMA in hair (pg/mg)	0.00 (±0.00)	1644.44 (±4859.92) n=6
MDEA in hair (pg/mg)	0.00 (±0.00)	10.00 (±42.43) n=1
MDA in hair (pg/mg)	0.00 (±0.00)	68.33 (±259.28) n=2

[a]Consumption per day/week captures the last six months, duration of use, and cumulative dose are averaged within the total group. Last consumption is averaged only for subjects who used the drug in the last six months. In this case, sample size is shown. The hair analysis was performed on two hair samples (each 3 cm in length) per participant capturing drug use over the last six months. Concentrations were averaged over the two samples. If the hair sample was not long enough, only one sample was analyzed (3 cm, 3 months). MDMA=3,4-methylenedioxy-N-methylamphetamine; methylenedioxymethamphetamine, MDEA=methylenedioxyethylamphetamine, MDA=3,4-methylenedioxyamphetamine.

4.4.2 ^{11}C-ABP688 uptake in controls, cocaine users, and smokers

In accordance with previous reports (Ametamey et al., 2007; DeLorenzo et al., 2011; Hefti et al., 2012), regional ^{11}C-ABP688 uptake was highest in mGluR5-rich regions such as the ACC, caudate, insula, putamen, MPFC, OFC, VLPFC, DLPFC, while ^{11}C-ABP688 binding was lower in the thalamus and substantially lower in the cerebellum.

^{11}C-ABP688 uptake did not differ between HC and CU (**Fig. 1A, 2A**), yet differed substantially between non-smokers (n=11) and smokers (n=24) (Fig. 1B, 2B). The mixed-effect ANOVA revealed significant main effects of *smoking status* [$F(1,31)=29.46$, $p<.0001$] and *brain region* [$F(11,341)=127.22$, $p<.0001$] but not of *group* [$F(1,31)=0.09$, $p=.77$] and *hemisphere* [$F(1,31)=0.86$, $p=0.36$]. Therefore, V_{norm} values of the left and right hemispheres were averaged for further calculations. Smokers showed marked decreases (14-21%) of mGluR5 availability in all VOIs compared to non-smokers (**Table 3**; **Fig. S1; Fig. S2**).

^{11}C-ABP688 uptake in all VOIs correlated significantly with age in CU (n=18, $r=.51$-$.58$, $p<.05$) and smokers [(n=24, 13 CU and 11 HC), $r=.41$-$.52$, $p<.05$; except for hippocampus and amygdala $p=.12$] but not in HC in general (n=17, $p>.88$). Moreover, ^{11}C-ABP688 uptake was significantly negatively associated with age in non-smokers in the putamen [(n=11, CU=5, HC=6), $r=-.65$, $p<.05$)].

4.4.3 Drug use parameters and ^{11}C-ABP688 uptake

In order to reduce the probability of α-error accumulation, three combined brain regions were derived by averaging the V_{norm} values of the OFC, ACC, MPFC, DLPFC, VLPFC (*Frontal cortex)*, the V_{norm} values of the caudate and the putamen (*Striatum)*, and the V_{norm} values of the amygdala, parahippocampaly gyrus, and the hippocampus (*Medial Temporal Lobe (MTL)*). The insula and the thalamus were not combined with other brain regions. To prevent inflating potential associations, for correlation analyses regarding drug use only users of the specific drug were included.

There were no significant associations of alcohol, cannabis, and cocaine use with mGluR5 availability (times/grams per week, last use, duration in years, cumulative dose in g), except for age of cocaine onset ($r_{Striatum\ VOI}=.47$, $p=.05$; $r_{MTL\ VOI}=.55$, $p<.05$; $r_{Thalamus\ VOI}=.48$, $p<.04$). However, when corrected for age, these correlations were not statistically significant anymore ($p>.30$). Weekly cigarette consumption and smoking duration did not correlate with mGluR5 availability, yet last nicotine use (hours) correlated strongly with mGluR5 availability ($r_{Frontal\ VOI}=.60$, $p<.01$; $r_{Striatum\ VOI}=.61$, $p<.01$; $r_{MTL\ VOI}=.57$, $p<.01$; $r_{Insula\ VOI}=.63$, $p<.01$; $r_{Thalamus\ VOI}=.57$, $p<.01$; **Fig. 3**). Moreover, age-of-onset of nicotine use was positively related to mGluR5 availability in the frontal VOI ($r=.41$, $p<.05$) and thalamus ($r=.45$, $p<.05$), but this result also did not survive correction for age ($p>.31$).

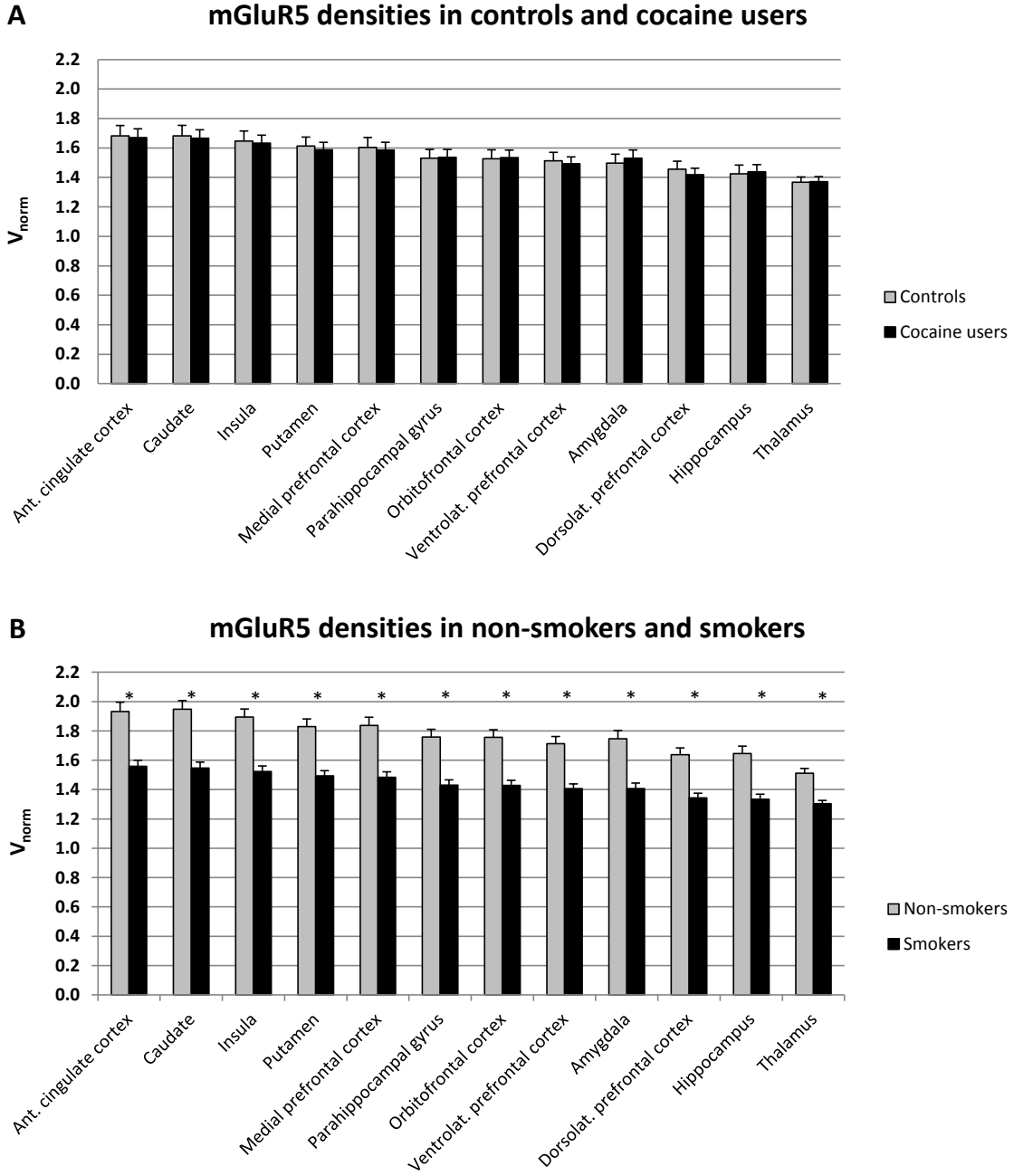

Fig. 1. Regional differences in mGluR5 density (means and standard errors). **(A)** Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake did not differ between controls (n=17) and cocaine users (n=18) in 12 predefined regions of interest. **(B)** Smokers (n=24) showed significantly decreased mGluR5 density in all regions of interest compared to non-smokers (n=11) irrespective of cocaine use. The percent difference of mGluR5 density in non-smokers and smokers ranged from 14 to 21 percent. *Indicates significant differences (p<.0001). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

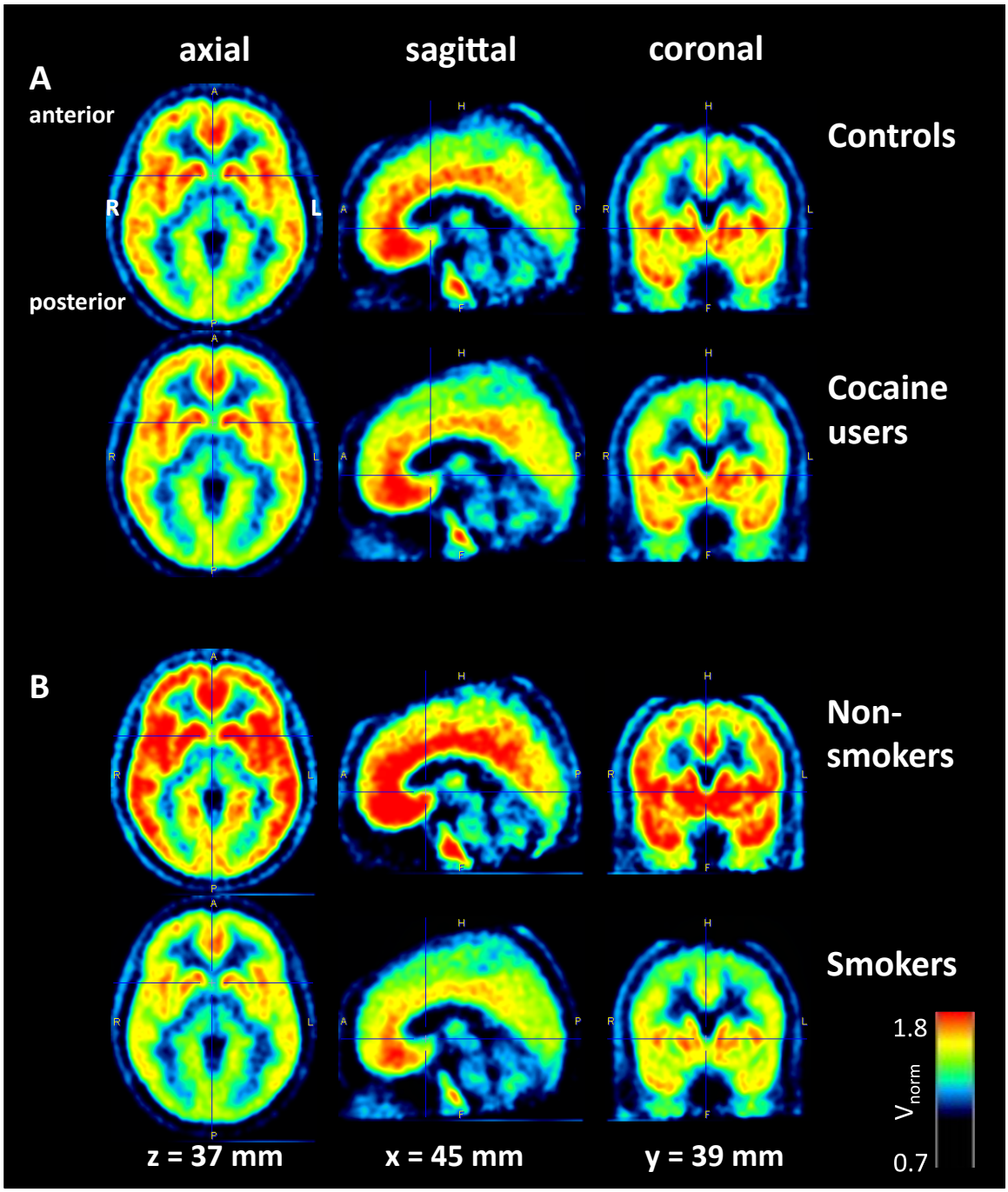

Fig. 2. Axial, sagittal, and coronal views of 11C-ABP688 binding. **(A)** ^{11}C-ABP688 binding did not differ between controls and cocaine users. **(B)** Smokers exhibited a marked global reduction of ^{11}C-ABP688 binding compared to non-smokers. V_{norm} = normalized volume of distribution. Crosshair position: (45, 39, 37; Montreal Neurological Institute brain atlas coordinates).

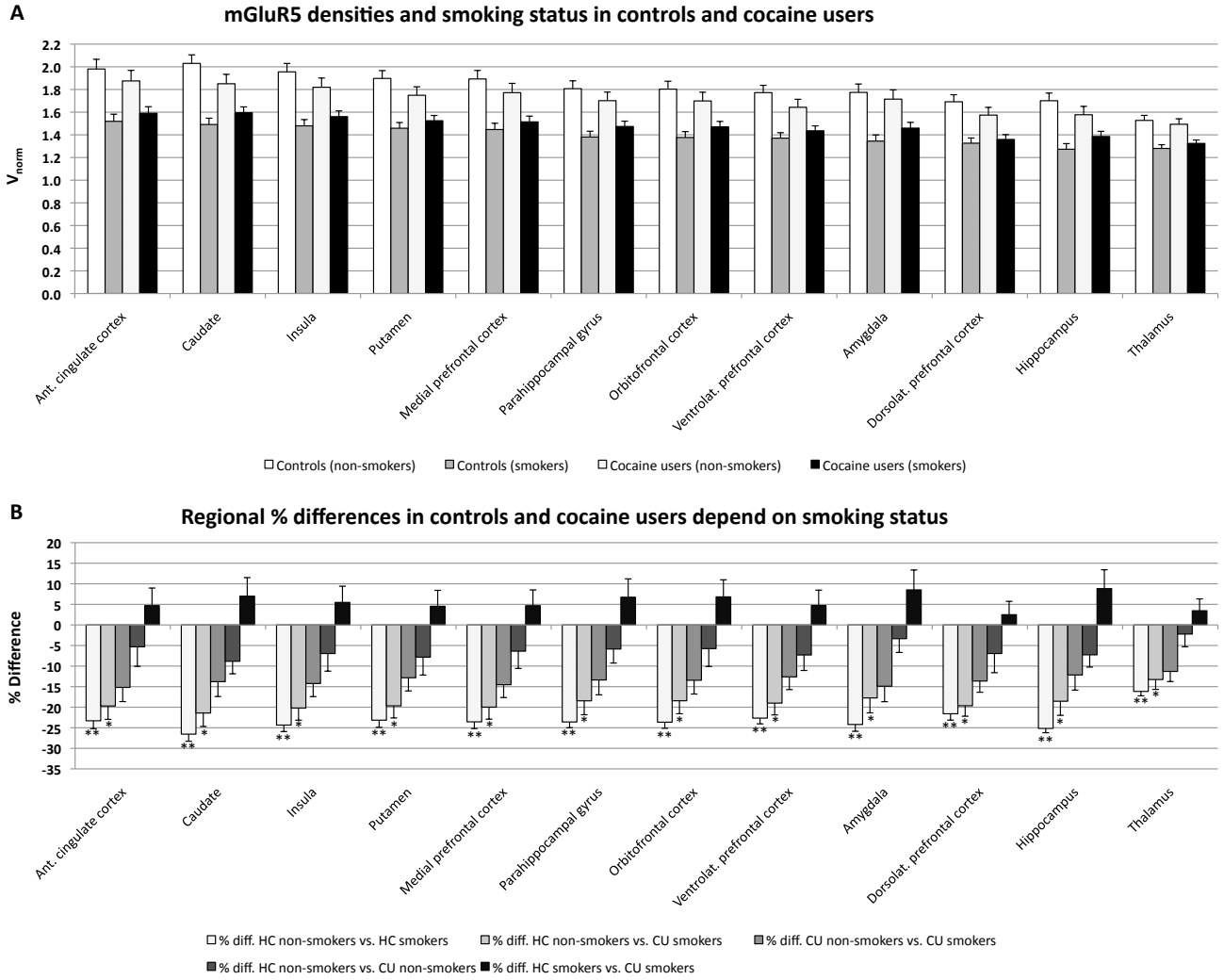

Fig. S1. Regional differences in mGluR5 density (means and standard errors) in controls and cocaine users depend on smoking status. **(A)** Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake differed significantly between non-smokers and smokers in all regions of interest irrespective of whether they used cocaine (a mixed-effect ANOVA revealed significant main effects of *group* [$F(3,31)=11.07$, $p<.0001$] and *brain region* [$F(11,341)=125.46$, $p<.0001$]). More specifically, smoking controls ($p<.0001$, Bonferroni-corrected post hoc comparisons) and smoking cocaine users ($p<.01$) had significantly lower mGluR5 density than non-smoking controls, and smoking controls ($p<.01$) and by trend smoking cocaine users ($p=.069$) had lower mGluR5 density than non-smoking cocaine users. **(B)** Percent difference in V_{norm} between non-smoking controls (n=6), smoking controls (n=11), non-smoking cocaine users (n=5), and smoking cocaine users (n=13). **Indicates significant differences ($p<.0001$), *indicates ($p<.01$). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

Table 3. Independent t-tests of age, nicotine use and brain regions for non-smokers and smokers (means and standard deviations)

	Nonsmokers (n = 11)	**Smokers (n = 24)**	***t*-value**	**df**	**% difference**	**Cohen's *d***
Age	38.09 (± 6.07)	35.33 (± 8.54)	-0.96	33	-	-
Cigarettes per week	0.00 (± 0.00)	101.83 (± 61.72)	8.08*	33	-	-
Smoking duration (years)	0.00 (± 0.00)	16.02 (± 7.94)	10.01*	33	-	-
Last nicotine use (hours)	-	73.73 (± 238.45)	-	-	-	-
Anterior cingulate cortex	1.93 (± 0.24)	1.56 (± 0.19)	-4.98	33	-19 ± 9.96%	1.39*
Caudate	1.95 (± 0.18)	1.55 (± 0.20)	-5.75	33	-21 ± 10.14%	1.50*
Insula	1.89 (± 0.21)	1.52 (± 0.17)	-5.53	33	-20 ± 9.04%	1.47*
Putamen	1.83 (± 0.18)	1.49 (± 0.17)	-5.42	33	-18 ± 9.17%	1.46*
Medial prefrontal cortex	1.84 (± 0.22)	1.48 (± 0.16)	-5.30	33	-19 ± 8.91%	1.44*
Parahippocampal gyrus	1.76 (± 0.16)	1.43 (± 0.18)	-5.24	33	-19 ± 9.96%	1.43*
Orbitofrontal cortex	1.76 (± 0.19)	1.43 (± 0.17)	-5.18	33	-19 ± 9.55%	1.42*
Ventrolater prefrontal cortex	1.71 (± 0.19)	1.41 (± 0.15)	-5.28	33	-18 ± 8.60%	1.44*
Amygdala	1.75 (± 0.17)	1.41 (± 0.19)	-5.08	33	-19 ± 10.82%	1.41*
Dorsolateral prefrontal cortex	1.64 (± 0.20)	1.34 (± 0.13)	-5.31	33	-18 ± 7.84%	1.44*
Hippocampus	1.64 (± 0.18)	1.33 (± 0.17)	-5.00	33	-19 ± 10.12%	1.39*
Thalamus	1.51 (± 0.11)	1.30 (± 0.10)	-5.30	33	-14 ± 6.91%	1.44*

*p<.0001

Eight out of 18 cocaine users tested positive for cocaine in the urine toxicology analysis. Therefore, an additional ANOVA was carried out where mGluR5 availability was compared among controls (n=17), cocaine users who tested negative (n=10), and cocaine users who tested positive for cocaine (n=8). There was no significant group difference for all VOIs indicating that recent cocaine use did not alter mGluR5 density in cocaine users. The same analysis was repeated with cannabis urine toxicology status, but recent cannabis use did also not affect our results. Cocaine craving scores did not correlate with mGluR5 availability in cocaine users.

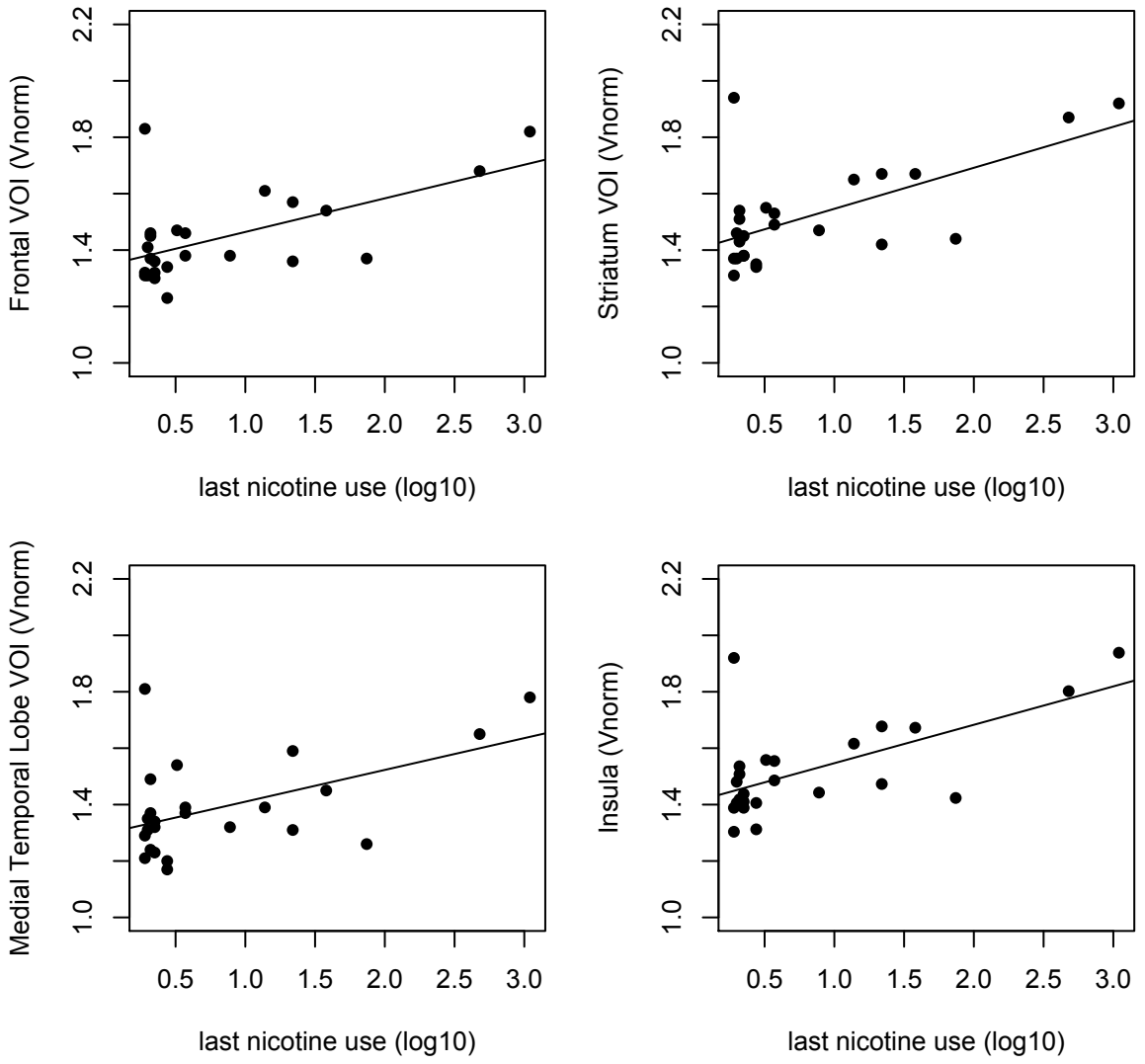

Fig. 3. Correlations between last nicotine use and mGluR5 availability in smokers (n=24). Longer duration of nicotine abstinence (hours) was associated with higher mGluR5 availability. V_{norm}, normalized volume of distribution of ^{11}C-ABP688; Frontal VOI, average of V_{norm} values of the OFC, ACC, MPFC, DLPFC, VLPFC; Striatum VOI, average of the V_{norm} values of the caudate and putamen; Medial Temporal Lobe VOI, V_{norm} values of the amygdala, parahippocampaly gyrus, and the hippocampus; r_p, Pearson's product-moment correlation coefficient.

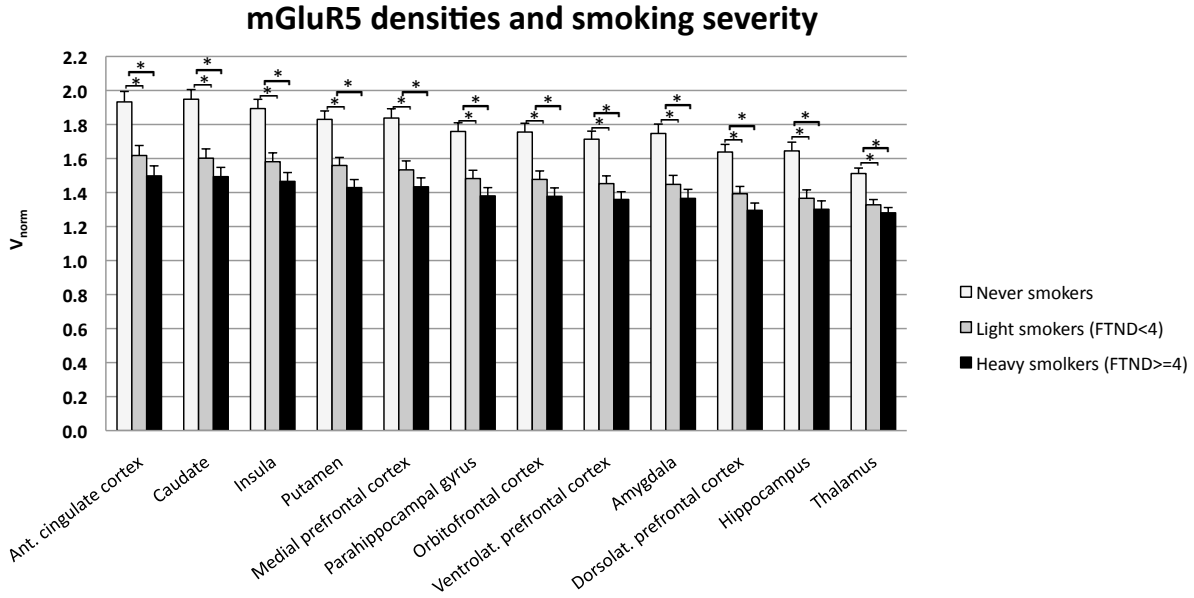

Fig. S2. Regional differences in mGluR5 density (means and standard errors) in never smokers (n=11), light smokers (n=12; FTND score <4) and heavy smokers (n=12; FTND score ≥4). Never smokers had significantly higher mGluR5 availability than light (p<.01) and heavy smokers (p<.0001), while light and heavy smokers did not significantly differ from each other (p=.47) (main effects of *group* [$F(2,32)=16.50$, p<.0001; linear contrast p<.0001] and *brain region* [$F(11,352)=125.19$, p<.0001] in mixed-effect ANOVA). FTND, Fagerström Test for Nicotine Dependence; V_{norm}, normalized volumes of distribution of ^{11}C-ABP688 uptake. **Indicates significant differences (p<.0001), *stands for (p<.01). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

4.5 Discussion

This is the first study to investigate mGluR5 availability in human cocaine users vs. drug-naïve healthy control subjects. The cocaine user sample was unique in terms of very little co-use of other illegal substances verified by hair toxicology analyses, and sparse psychiatric co-morbidities. The key findings are that cocaine use was not associated with altered mGluR5 density because no regional differences of ^{11}C-ABP688 binding between cocaine users and controls emerged. Moreover, the cocaine use parameters were not associated with mGluR5 availability. In contrast, smokers exhibited marked global decreases with an average of 20% in mGluR5 density compared to non-smokers. The time interval since the last nicotine use was strongly related to mGluR5 density in all regions of interest, reflecting that the decrease of mGluR5 availability was particularly pronounced in individuals who had smoked very recently. Interestingly, while older age was associated with higher mGluR5 availability in cocaine users and smokers, an opposing trend was observed in non-smokers, whereas no significant correlations between age and mGluR5 availability were found in controls.

With the present study, we clearly replicated the recent finding that nicotine use is associated with a marked decrease in ^{11}C-ABP688 uptake (Akkus et al., 2013) representing either altered affinity of the binding site or decreased mGluR5 density. Because G-protein coupled receptors have the ability to undergo endocytosis in response to changes in extracellular levels of receptor agonists (Gainetdinov et al., 2004), the notion that the strong mGluR5 decrease of 20% in smokers constitutes a mechanism to protect the receptors from nicotine-triggered glutamatergic over-stimulation by down-regulation or internalization seems probable (Dhami and Ferguson, 2006; Fourgeaud et al., 2003; Trivedi and Bhattacharyya, 2012). Indeed, the fact that longer nicotine abstinence was associated with increased mGluR5 availability may reflect the dynamic nature of mGluR5 trafficking and support the assumption of nicotine-induced changes. Alternatively, reduced mGluR5 availability may constitute a pre-existing condition that increases the risk for nicotine dependence. The average mGluR5 reduction (20%) observed in our study was remarkably in accordance with recent data (Akkus et al., 2013). However, although mGluR5 reductions were slightly more pronounced in the caudate compared to other brain regions in smokers, we did not find a strong region-specific decrease as was previously revealed for the medial OFC in smokers (Akkus et al., 2013). Moreover, cigarettes smoked per week and severity of nicotine dependence in our study did also not correlate with mGluR5 availability. Furthermore, we also failed to find a significant association between duration of nicotine use and mGluR5 availability. However, when Akkus et al. (Akkus et al., 2013) took age into account, the association was not significant as well. A notable difference emerged with regard to nicotine abstinence, where we found that shorter nicotine abstinence was associated with decreased mGluR5 availability. This divergent finding is likely explained by the fact that smokers exhibited a larger variance of abstinence periods in our study. Of further interest is the concurring finding that in both studies older age was significantly associated with increased mGluR5 binding in smokers and in our study also in cocaine users but not in control subjects. In fact, we found an opposing trend indicating that older age was associated with decreased mGluR5 availability in non-smokers.

Preclinical studies have shown that chronic self-administration of cocaine disrupts synaptic communication between the PFC and the striatum and results in decreased basal levels of non-synaptic, extracellular glutamate in the NAcc (Baker et al., 2003; Kalivas and Brady, 2012; Kalivas and Volkow, 2005; Madayag et al., 2007; McFarland et al., 2003; Miguens et al., 2008; Pierce et al., 1996). Reduced extracellular glutamate tone in the NAcc has in turn been associated with down-regulation of mGluR5 expression and function (mainly in the NAcc core) in rats (Ary and Szumlinski, 2007; Ben-Shahar et al., 2009; Fourgeaud et al., 2004; Ghasemzadeh et al., 2009; Hao et al., 2010; Swanson et al., 2001), resulting in disrupted plasticity, which is likely to contribute to the persisting nature of addiction (Kalivas, 2009). Although the exact function of mGluR5s in addiction is not entirely understood, they have directly been linked to drug-seeking behavior and extinction learning in animals withdrawn after cocaine self-administration (for reviews Bellone and Mameli, 2012;

Duncan and Lawrence, 2012; Kenny and Markou, 2004; Olive, 2010). Accordingly, mGluR5 null mutant mice neither self-administered cocaine nor exhibited increased locomotor activity after cocaine treatment (Chiamulera et al., 2001) and self-administration and reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin is attenuated by mGluR5 antagonists (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Kenny et al., 2005; Kumaresan et al., 2009; Lee et al., 2005; Martin-Fardon et al., 2009; Paterson and Markou, 2005; Paterson et al., 2003; Platt et al., 2008; van der Kam et al., 2007). Moreover, it has been suggested that genetic variations in mGluR5s, resulting in decreased mGluR5-mediated neurotransmission, may render individuals less sensitive to the reinforcing effects of cocaine and nicotine, as well as aversive states during withdrawal as the chromosomal region 11q14, on which the metabotropic glutamate receptor 5 (GRM5) gene is located has been associated with habitual smoking behavior (Bierut et al., 2004; Stoker et al., 2012). Several explanations have been proposed how mGluR5s modulate cocaine-mediated behaviors and which mechanisms underlie the effectiveness of pharmacological antagonism, however, more research is required to confirm them. One suggestion has been that in the NAcc interactions of mGluR5s with the Homer family, post-synaptic scaffolding proteins influencing mGluR5 trafficking and signal transduction, may contribute to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking changes (Kumaresan et al., 2009). Furthermore, it has been shown that a single exposure to cocaine can lead to reduced expression of mGluR5s, which may transiently impair mGluR5-dependent long-term depression (LTD) mediated via activation of cannabinoid 1 receptors (Fourgeaud et al., 2004; Luscher and Huber, 2010). It has been proposed, that the observed down-regulation of mGluR5s and Homer1b/c after chronic cocaine administration may constitute a compensatory mechanism, as mGluR5 antagonists attenuate drug-seeking behaviors (Kalivas, 2009). Interestingly, extinction training was also associated with a marked decrease in mGluR5 expression and elevated levels of Homer proteins, possibly providing further support for compensatory adaptation, which may inhibit relapse (Kalivas and Volkow, 2011).

Even though findings from preclinical and human studies are not directly comparable, it is noteworthy that preclinical cocaine studies also failed to find alterations in mGluR5 expression in the PFC and the amygdala, which is consistent with our study (Ben-Shahar et al., 2009; Hao et al., 2010; Schmidt et al., 2011; Swanson et al., 2001). Although cocaine users of the present study did not differ from controls in mGluR5 availability in any of the investigated brain regions, we cannot fully exclude the possibility that cocaine users may have nevertheless exhibited subtle alterations in mGluR5 availability in the NAcc core region. Due to limited spatial resolution we were unable to investigate the NAcc with its core and shell subcomponents separately in a reliable manner. Moreover, the lack of surface expression changes in cocaine users does not necessarily mean that the function of mGluR5 was unaffected. In addition, there was no significant relationship between cocaine craving and

mGluR5 availability. However, reported craving urges were rather low and no cocaine-associated cues were presented to cocaine users that could have elicited stronger craving urges.

The question arises why cocaine use did not seem to alter mGluR5 availability while nicotine use did so in a very pronounced and global manner. We can only speculate about underlying reasons but in contrast to cocaine, nicotine has the ability to increase glutamate release by directly binding to nicotinic acetylcholine receptors that are located on presynaptic terminals of glutamatergic neurons in the PFC, VTA, NAcc, amygdala, and the hippocampus (Mansvelder and McGehee, 2002) and may thereby result in the specific effect we observed in smokers in the present study. In contrast, cocaine binds to the dopamine transporters (as well as norepinephrine and serotonin transporters), which are mainly localized in the striatum, to some extent in substantia nigra, and to very low or no extent in the neocortex (Hall et al., 1999). Acute cocaine intake may therefore exert its function on glutamatergic transmission in a more indirect and less global manner compared to nicotine. Finally, in agreement with our findings, preclinical studies demonstrated specific alterations of mGluR5 expression primarily in the NAcc core rather than global changes (Ary and Szumlinski, 2007; Ben-Shahar et al., 2009; Fourgeaud et al., 2004; Ghasemzadeh et al., 2009; Hao et al., 2010; Swanson et al., 2001).

Some limitations of the present report merit comment: i) Due to limited spatial resolution of PET imaging, we cannot fully exclude the possibility that cocaine users may have exhibited subtle differences especially in the NAcc core region (or other small regions) in comparison to controls. ii) The present study cannot conclusively answer if the reduced mGluR5 availability in nicotine users represents a pre-existent condition or is indeed acquired. iii) A PET study using the radioligand ^{18}F-FPEB reported that the human cerebellum is not entirely devoid of mGlu5 receptors (Patel et al., 2007). However, a more recent study with ^{18}F-FPEB demonstrated that using the cerebellum as a reference region may be feasible to quantify mGluR5 density (Barret et al., 2010). Additional support for negligible mGluR5 binding in the cerebellum comes from prior in vitro and in vivo studies with the radioligand ^{11}C-ABP688 (Elmenhorst et al., 2010; Hamill et al., 2005). Lastly, no mGluR5 protein expression was detected in the cerebellum in a human postmortem study using Western blotting (Deschwanden et al., 2011). iv) The strong influence of recent nicotine use may have masked less pronounced cocaine-related effects on mGluR5 expression. However, it is difficult to recruit cocaine-addicted individuals who do not smoke and cocaine users and controls were well-matched for nicotine use.

In conclusion, this is the first study to show that specifically nicotine but not cocaine use was associated with a marked global decrease of 20% in mGluR5 density. In particular, shorter nicotine abstinence duration was associated with lower mGluR5 density pointing to the possibility that mGluR5 might be nicotine-induced. These findings provide evidence that mGluR5s are involved in nicotine addiction and may have important implications with regard to the development of potential pharmacotherapies aimed at facilitating smoking cessation. If compounds targeting mGluR5s are effective in preventing relapses in human cocaine users akin to preclinical findings remains to be determined. Since the systematic investigation of neuroadaptations in humans is much more complicated due to constraints in experimental manipulation that is only possible in preclinical research, it would be informative to apply multimodal imaging in human cocaine users. For instance free glutamate levels in the NAcc and PFC measured by means of magnetic resonance spectroscopy and how these levels are related to mGluR5 availability, PFC metabolism, and resting state activity in different stages of addiction (e.g., acute drug intake/relapse, craving, withdrawal) could be insightful to unmask neuroadaptations in drug-addicted humans. Lastly, it is currently not clear if simultaneous cocaine and nicotine use has an additive effect in the transition from controlled to addicted drug use by leading to more pronounced neuroadaptations, and if abstinence from either substance is more difficult to achieve due to co-use.

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4.7 Supplemental information

4.7.1 Methods

Methodology of the Urine Analysis

Urine toxicology analysis comprised the compounds/substances tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and was assessed by a semi-quantitative Enzyme Multiplied Immunoassay method (Dimension RXL Max, Siemens, Erlangen, Germany) (1).

Methodology of the Hair Analysis

If participants' hair was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine, MDMA, MDEA, MDA, morphine, codeine, methadone EDDP (primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis a three step washing procedure with water (2 minutes shaking, 15ml), acetone (2min., 10ml) and finally hexane (2min., 10ml) of hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5ml, 16hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33 % (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5. MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of HPLC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge,

(Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1mM ammonium formate buffer adjusted to pH 3,5 with formic acid (eluent A) and acetonitrile containing 1mM ammonium formate and 1 mM formic acid (eluent B). The Analysis was performed in MRM mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

4.7.2 Reference

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5

OPPORTUNISM AND IMMEDIACY BIAS IN RECREATIONAL AND DEPENDENT COCAINE USERS

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Personal contribution

LMH collected and analyzed the data, interpreted the data, and wrote the manuscript. CE helped to design the economic decision-making task and revised the first draft of the manuscript. KHP, MV, and ES contributed to data acquisition and/or revised the first draft of the manuscript. KB contributed the economic decision-making tasks and revised the first draft of the manuscript. MRB contributed hair toxicology analyses. BBQ conceived and designed the study, interpreted the data, and revised the first draft of the manuscript.

5.1 Abstract

Background. Maladaptive decision-making is a core feature of cocaine addiction. Imaging studies have demonstrated that dependent cocaine users manifest changes in brain areas associated with decision-making including medial and orbital prefrontal cortex – regions which are also involved in many socio-cognitive processes. However, these functions have not been examined in cocaine users yet. Moreover, it is unknown if even recreational and non-dependent cocaine use is linked to decision-making deficits. Therefore, we investigated whether recreational and dependent cocaine users exhibit alterations in social and non-social decision-making.

Method. The performance of stimulant-naïve controls (n=68), recreational (n=68) and dependent cocaine users (n=30) in classical decision-making paradigms (Iowa Gambling Task and Delay Discounting) and in social interaction paradigms (Distribution Game and Dictator Game) was assessed.

Results. Decisions in the social interaction tasks of both cocaine user groups were more self-serving compared to controls. In the Iowa Gambling Task, only dependent cocaine users performed worse than controls. They were also more likely to choose immediate smaller rewards over larger delayed rewards in the Delay Discounting.

Conclusions. Our results imply that both recreational and dependent cocaine users are more concerned with their own monetary gain when interacting with another person. Furthermore, primarily dependent cocaine users are less foresighted and more impulsive regarding immediate reward. Overall, social interaction deficits are already present in recreational users, while non-social decision-making deficits occur predominantly in dependent cocaine users. Thus, social interaction training and cognitive remediation strategies may improve treatment success and quality of life in dependent cocaine users.

5.2 Introduction

Cocaine is the second most used illegal drug in Europe after cannabis and it is estimated that 14.5 million Europeans have tried cocaine at least once in their life, amounting to a lifetime prevalence of 4.3% (EMCDDA, 2011). Moreover, cocaine is the primary illegal drug responsible for drug-dependence treatment in North and South America (United Nations Office on Drugs and Crime, 2011). Although not everyone will undergo the transition to dependence, cocaine is classified as a highly addictive drug (Nutt et al., 2007), and it is estimated that around 21% of cocaine users will meet dependence criteria by the age of 45 years (Wagner and Anthony, 2002). As evidenced by epidemiological data, a substantial number of non-addicted recreational cocaine users (RCU) exist as well (EMCDDA, 2011). The health risks associated with cocaine abuse include severe medical complications, such as cardiovascular or respiratory incidences, and a number of psychiatric disorders (Buttner, 2012). Because drug addiction results in high economic and societal costs (Olesen et al., 2012), and effective pharmacological treatment options are currently lacking (O'Brien, 2005), an adequate characterization of the core feature of cocaine addiction, maladaptive decision making, is crucial for the development of effective prevention and treatment strategies.

Decision-making deficits in addicted individuals are well captured by the paradox that they compulsively seek and take the drug despite encountering adverse legal, financial, health-related, and social consequences (Koob, 2009). Accordingly, studies with dependent cocaine users (DCU) reported that reductions in striatal dopamine D_2 receptor availability were related to decreased metabolic activity in the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) (Volkow et al., 1993), brain regions which are crucially involved in autonomous decision-making and in social interaction (Goldstein, and Volkow, 2011; Lucantonio et al., 2012; Rilling and Sanfey, 2011). In fact, anecdotal reports suggest that there might be deficits in social interaction associated with cocaine abuse. In a study with crack-cocaine dependent individuals decision-making deficits were associated with real-life social dysfunction (Cunha et al., 2011). However, in this study social dysfunction was solely based on a self-report scale. Moreover, cocaine users feature a 22-fold increased risk for an antisocial personality disorder and clinical reports have given account of egocentrism and blunted emotion in cocaine-dependent individuals (Rounsaville, 2004). Though, adequate social cognitive abilities (e.g., empathy, emotion recognition, theory of mind) are known to have a strong impact on development, course, and outcome of psychiatric diseases (Couture et al., 2006) and may also affect the course of dependence and treatment success in stimulant abusers (Homer et al., 2008). Thus, knowing potential effects of cocaine addiction on social interaction is fundamentally important, but to date, there are no data available about whether these social impairments can be captured in a controlled laboratory environment using measures of human social interaction.

Because the addiction process is not dichotomous but rather gradual, advancing from habitual to compulsive use (Haber, 2008), *recreational* cocaine use can be thought of as reflecting an intermediate step in addiction. Preliminary studies suggest that RCU display impaired cognitive functions such as response inhibition, which was proportional to lifetime cocaine exposure indicating a cumulative effect (Colzato, 2007, 2009). Additionally, we recently reported deficits in early information processing and blue-yellow colour vision in RCU suggesting alterations of catecholamine neurotransmission at an early stage of cocaine abuse (Hulka et al., 2012; Preller et al., 2012). Thus, basic cognitive and neurobiological mechanisms are already impaired in recreational users, but whether these extend to decision-making is presently unclear. Experimental studies in DCU have provided evidence that chronic cocaine use is linked to deficits in the processing of reward and punishment contingencies, as measured by the Iowa Gambling task (IGT) (Bechara et al., 2002; Kjome et al., 2010; Verdejo-Garcia et al., 2007*a*) and a relative inability to gratify delayed rewards (Bickel et al., 2011*a*; Heil et al., 2006; Kirby and Petry, 2004). The latter is particularly relevant as it is associated with negative outcomes in the financial, academic, and health domain (Mischel et al., 2011) and poor treatment response in cocaine dependent individuals (Washio et al., 2011). However, although these studies are suggestive of similar, but weaker deficits in RCU, this has not been tested so far.

The concomitant intake of other drugs of abuse (i.e., polytoxic drug abuse) is one of the major confounding factors in addiction research, while the reliability of self-reported data has been questioned (Hser, 1997). Analyzing urine samples is a more objective option, but only allows the detection of drug use over the past few days. It does not allow testing of whether participants have used drugs other than cocaine *in the last months*. An elegant way to achieve this is to perform toxicological hair analyses. Thus, a combination of self-reported drug use and objective measures is clearly warranted.

In sum, in this cross-sectional study, we investigate social and non-social decision-making behaviour of RCU and DCU in comparison to an age, sex, and IQ matched healthy control group. We report on effects of cocaine abuse on measures of selfishness and extend on previous reports in the domain of risk taking and discounting of delayed rewards by incorporating a group of RCU. We hypothesize that RCU exhibit similar but less pronounced behavioral changes than DCU. Furthermore, as psychiatric co-morbidities such as Attention-Deficit/Hyperactivity Disorder (ADHD) and depression are frequently present among addicted individuals (Ivanov et al., 2008; Perez de Los Cobos et al., 2011; Rounsaville, 2004), we conducted a comprehensive psychiatric diagnostic interview and assessed ADHD and depressive symptoms in both RCU and DCU. Finally, we report a unique, comprehensive set of data on self-reported and objective measures of drug use in our sample.

5.3 Materials and methods

5.3.1 Participants

The present sample represents the cross-sectional part of the longitudinal Zurich Cocaine Cognition Study (ZuCo^{2}St) and consists of 68 RCU, 30 DCU, and 68 psychostimulant-naïve control subjects (total n=166). Details regarding recruitment, selection process and study procedure are provided in the **Supplement Text**. Inclusion criteria for the two cocaine user groups were cocaine as primary drug, cocaine use of >0.5g per month, and an abstinence duration of <6 months. Cocaine dependence was diagnosed according to the Diagnostic and Statistical Manual-IV (DSM-IV) criteria, with only DCU meeting these criteria. All participants had to be aged between 18 to 65 years and proficient in German.

Exclusion criteria were use of opioids or prescription drugs affecting the CNS, presence of a current or previous Axis I DSM-IV psychiatric disorder (other than cocaine and alcohol abuse/dependence, ADHD, and a former affective disorder), neurological disorders or head injury, and a family history of a severe DSM-IV psychiatric disorder such as schizophrenia, bipolar disorder or obsessive-compulsive disorder. Participants were instructed to abstain from illegal drugs for a minimum of three days and from alcohol for at least 24 hours.

Urine samples were collected to control for recent drug use. To characterize drug use over the last six months objectively, hair samples were collected and analyzed with liquid chromatography-mass spectrometry (**Supplement Text**). Participants received financial compensation of 170-225 Swiss Francs, depending on their performance or decisions in some of the tasks. The study was approved by the Cantonal Ethics Committee of Zurich and all participants provided written informed consent.

5.3.2 Clinical interviews and questionnaires

The *Structured Clinical Interview for DSM-IV Disorders* was carried out by trained psychologists. To estimate pre-morbid verbal intelligence the *Mehrfachwahl-Wortschatz-Intelligenztest* (MWT-B) was applied (Lehrl, 1999). Drug use was assessed by means of the *Interview for Psychotropic Drug Consumption*, which has been described in detail elsewhere (Quednow et al., 2004). The brief version of the *Cocaine Craving Questionnaire* (CCQ) was used to assess current cocaine craving (Sussner et al., 2006; Tiffany et al., 1993). The *Attention Deficit Hyperactivity Disorder Self-Rating* scale (ADHD-SR) enabled the diagnosis of ADHD in adulthood (Rosler et al., 2004). Symptoms of depression were measured by means of the *Beck Depression Inventory* (BDI) (Beck et al., 1961).

5.3.3 Behavioral tasks

Social decision-making: Participants' social preferences were assessed in a Distribution Game followed by a Dictator Game (Charness and Rabin, 2002; Engelmann and Strobel, 2004) implemented in z-Tree (Fischbacher, 2007). The Distribution Game involves two players, player A and B. Player A chooses one of ten possible point distributions ranging from a fair distribution where both players would receive 25 points each to the most opportunistic distribution where player A would receive 40 points and player B one point. Player B is a passive recipient and is merely informed about which distribution player A chose and how many points both players receive. In addition, the Distribution Game also allows classification of subjects according to their efficiency preferences. A subject that is motivated by efficiency concerns values the total monetary payoff for the dyad positively. Participants were classified as *fair* when they chose the first distribution, as *unfair and efficient* when they chose distributions two to five (yielding the highest total payoff), and as *unfair and inefficient* when they selected distributions six to ten (resulting in lower overall payoff for the dyad; **Fig. S1**). The Dictator Game always followed the Distribution Game, and the participants were told that they would play with another player B. Player A receives an endowment of 50 points and can give any amount from 0 to 50 points to player B. All subjects received a payment according to the points earned in the decision-making tasks. In both tasks, each point earned was worth CHF 0.25. Subjects received payment in cash in private at the end of the experiment or via online banking.

A

Distribution	Payoff X	Payoff Y	Total Payoff
Fair			
1	25	25	50
Unfair efficient			
2	29	24	53
3	32	23	55
4	34	22	56
5	35	21	56
Unfair inefficient			
6	36	19	55
7	37	16	53
8	38	12	50
9	39	7	46
10	40	1	41

B

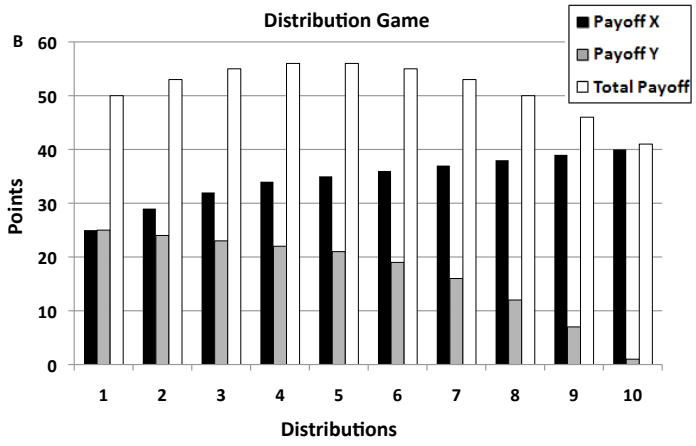

Fig. S1. *(A)* depicts the point values of the payoffs for participant A and B, and the total payoffs for the ten distributions in the Distribution Game. Participants can be classified into three categories according to their preferences for efficiency. Participants are *fair* when choosing distribution 1 where participant A and B both receive 25 points each; *unfair efficient* when choosing distributions 2-5, yielding the highest total payoffs; and *unfair inefficient when* choosing distributions 6-10, resulting in the destruction of money. *(B)* illustrates point values of the payoffs for participant A and B, and the total payoffs for the ten distributions.

Non-Social Decision-Making: We tested participants' risk-taking preferences and planning abilities using the IGT, which has been described in detail before (Bechara et al., 2002; Quednow et al., 2007). Intertemporal choice was measured using the DD according to Kirby et al (1999). Further details of the tasks are given in the Supplement.

5.3.4 Statistical analysis

Statistical analyses were performed with PASW 19.0 (SPSS Inc.). We computed two domains: the Social (SDM) and Nonsocial Decision-Making (NSDM) domains by averaging z-transformed measures derived in the subtasks. Z-transformation was carried out on the basis of means and standard deviations of the control group. For the SDM domain, we used the following dependent variables: "payoff for B" in the Distribution Game and the "payoff for B" in the Dictator Game. The NSDM domain comprised of the "total ratio of favorable and unfavorable cards chosen in the IGT" and the "overall discounting parameter (k)" in the DD. We then performed dummy coded multiple regression analyses to explore relevant predictors for the behavioral tasks. Additional repeated-measure analyses of covariance (ANCOVAs) were conducted for the IGT and DD. Correlation analyses (Pearson's product-moment) were calculated to relate drug use parameters to the performance in the behavioral tasks. As the assumptions of homoscedasticity and parametric distribution were not met by some variables, the drug use variables grams per week and lifetime use in grams were log-transformed (log10) and the constant 1 was added because the data contained 0 values. Demographic data were analyzed by means of analyses of variance (ANOVA) with Sidak-corrected *post hoc* analyses and by means of frequency analyses (Pearson's Chi^2 test).

5.4 Results

5.4.1 Demographic variables

Groups did not differ regarding demographic variables except for years of education (**Table 1**; **Table S1** for socioeconomic status data). RCU and DCU did not differ from controls regarding age, but DCU were by trend slightly older than RCU. Moreover, there were marginally, but not significantly, more males in the cocaine user groups compared to the control group and a previous study has reported sex differences in IGT performance (Bolla et al., 2004). Therefore, we introduced years of education, age, and sex as covariates in all statistical models. As RCU and DCU both reported more symptoms of ADHD and depression than controls (**Table 1**), additional analyses were conducted to examine a potential influence of these factors on decision-making.

Table 1. *Demographic data (means and standard deviations, number of subjects and percent)*

	Stimulant-naïve controls (n=68)	Recreational cocaine users (n=68)	Dependent cocaine users (n=30)	Value[a]	*P*[a]	df
Age	30.63 (±9.15)	28.71 (±6.19)	32.80 (±9.54)	2.77	0.07	2
Sex (m, f)	46, 22 (68, 32 %)	50, 18 (73, 27 %)	22, 8 (73, 27 %)	0.66	0.72	2
Years of education	10.61 (±1.77)	10.50 (±1.96)*	9.48 (±1.19)*	4.60	**0.01**	2
Verbal IQ (MWT-B)	104.66 (±10.41)	103.21 (±9.58)	100.93 (±12.01)	1.36	0.26	2
Smokers/Nonsmokers	54, 14 (79, 21 %)	61, 7 (90, 10 %)	27, 3 (90, 10 %)	3.50	0.17	2
ADHD-SR	7.84 (±4.71)	13.16 (±8.98)**	17.00 (±8.85)**	17.60	**0.00**	2
Beck Depression Inventory	4.41 (±4.38)	7.35 (±6.14)*	11.8 (±8.58)**††	15.85	**0.00**	2
Cocaine Craving Questionnaire	-	19.04 (±9.10)	20.90 (±11.68)	-0.85[b]	0.40	96

[a]ANOVA (all groups) or Chi^2-test (all groups) for frequency data, [b]T-test (only cocaine user groups).
*indicates significant post-hoc test (Sidak) vs. control group: $p<.05$, **p<.01.
††indicates significant post-hoc test (Sidak) vs. recreational cocaine user group: $p<.01$.

Table S1. *Socioeconomic status (number of subjects and percent)*

	Stimulant-naïve controls (n=68)	Recreational cocaine users (n=68)	Dependent cocaine users (n=30)	Value	*P*	df
0 - 15'000 CHF	26 (38.20 %)	18 (26.50 %)	12 (40.00 %)	2.75	0.25	2
15'000 - 30'000 CHF	15 (22.10 %)	11 (16.20 %)	10 (33.30 %)	3.62	0.16	2
30'000 - 60'000 CHF	12 (17.60 %)	20 (29.40 %)	4 (13.30 %)	4.28	0.12	2
60'000 - 90'000 CHF	10 (14.70 %)	16 (23.50 %)	2 (6.70 %)	4.61	0.10	2
90'000 - 120'000 CHF	2 (2.90 %)	2 (2.90 %)	1 (3.30 %)	0.01	0.99	2
120'000 CHF and more	3 (4.40 %)	1 (1.50 %)	1 (3.30 %)	1.02	0.60	2

Participants were asked how much money they had available over the past year. Chi^2-test.

5.4.2 Self-reported and objective drug use

Self-reported drug use showed that RCU used cocaine on a regular basis with a mean weekly consumption of about 1g of cocaine but did not meet DSM-IV criteria for cocaine dependence. Several participants tested positive for cocaine and cannabis in the urine toxicology but we decided not to exclude them in order to investigate potential effects of recent drug use (**Table 2**).

Table 2. *Pattern and amount of self-reported drug use: Results of the Psychotropic Drug Interview (means and standard deviations)*

	Stimulant-naïve controls (n=68)	**Recreational cocaine users (n=68)**	**Dependent cocaine users (n=30)**
Nicotine			
Cigarettes per day	9.29 (±9.73)	11.7 (±8.77)	16.05 (±13.77)
Years of use	9.61 (±9.54)	9.65 (±6.37)	13.55 (±8.54)
Alcohol			
Grams per week	110.49 (±120.21)	167.8 (±117.47)	199.7 (±259.4)
Years of use	13.62 (±9.38)	11.23 (±5.07)	12.89 (±8.64)
Cocaine			
Times per week	0.00 (±0.00)	1.07 (±1.03)	2.93 (±2.53)
Grams per week	0.00 (±0.00)	1.11 (±1.41)	6.17 (±8.70)
Years of use	0.00 (±0.00)	6.47 (±3.99)	9.22 (±6.43)
Maximum dose (grams/day)	-	3.46 (±2.47)	8.75 (±7.86)
Cumulative dose (grams)	0.00 (±0.00)	519.69 (±751.23)	4619.94 (±8658.35)
Last consumption (days)	-	27.45 (±37.6) n=68	20.43 (±33.78) n=30
Urine toxicology (pos./neg.)	0, 100 (0, 100%)	10, 57 (15, 85%)	13, 17 (43, 57%)
Amphetamine			
Grams per week	0.00 (±0.00)	0.08 (±0.21)	0.05 (±0.19)
Years of use	0.01 (±0.00)	1.63 (±2.97)	1.54 (±3.16)
Cumulative dose (grams)	0.00 (±0.00)	21.19 (±56.77)	22.26 (±62.80)
Last consumption (days)	-	90.46 (±145.48) n=24	78.38 (±75.42) n=6
MDMA			
Pills per week	0.00 (±0.00)	0.08 (±0.25)	0.41 (±1.83)
Years of use	0.25 (±1.64)	2.47 (±3.76)	3.06 (±5.22)*
Cumulative dose (pills)	0.73 (±2.75)	35.86 (±90.47)	157.38 (±393.52)
Last consumption (days)	-	124.91 (±167.18) n=21	82.13 (±45.43) n=9
Cannabis			
Grams per week	0.53 (±1.50)	0.86 (±2.05)	1.22 (±3.74)
Years of use	4.68 (±6.63)	7.74 (±6.03)	9.54 (±8.94)
Cumulative dose (grams)	479.16 (±1083.03)	1042.85 (±1780.04)	2626.67 (±3857.12)
Last consumption (days)	39.02 (±50.42) n=29	22.44 (±32.57) n=43	72.75 (±211.62) n=18
Urine toxicology (pos./neg.)	9, 59 (13, 87%)	12, 55 (18, 82%)	9, 21 (30, 70%)
Serotonergic Hallucinogens			
Cumulative dose (times)	0.80 (±2.17)	6.03 (±14.59)	5.75 (±10.47)
Last consumption (months)	97.57 (±93.54) n=14	66.24 (±61.18) n=29	181.99 (±339.56) n=18
GHB			
Cumulative dose (times)	0.00 (±0.00)	1.76 (±9.48)	1.28 (±2.89)
Last consumption (months)	-	126.07 (±31.37) n=3	30.00 n=1

Means and standard deviations in parenthesis. Consumption per day/week captures the last six months, duration of use and cumulative dose are averaged within the total group. Last consumption is averaged only for subjects who used the drug in the last six months. In this case, sample size is shown. MDMA = 3,4-methylenedioxy-N-methylamphetamine. Hallucinogens = psilocybin, lysergic acid diethylamide (LSD), N,N-Dimethyltryptamine (DMT), 3,4,5-trimethoxyphenethylamine (mescaline), 4-bromo-2,5-dimethoxyphenethylamine (2-CB). GHB = γ-Hydroxybutyric acid.

Results from the hair toxicology analyses revealed that self-reported cocaine use (g/week, cumulative dose, duration of use) corresponded with concentrations of cocaine and its metabolites in the hair samples (r=.29-.41, all p<.01). Importantly, hair toxicology provided evidence that the RCU and DCU enrolled in the present study are unique with regard to three crucial aspects (**Table 3**): 1) For both drug user groups, cocaine had been the main drug of use over the past six months. As expected, concentrations of cocaine and its metabolites were 7-fold higher in DCU than in RCU. 2) RCU and DCU did not differ significantly with regard to concentrations of amphetamines, methylphenidate, MDMA, and opiates. 3) For both RCU and DCU, concentrations of amphetamines and opiates were below the recommended cut-off value of 200 pg/mg (Cooper et al., 2012), indicating no regular use of these drugs over the past six months. Although the MDMA concentrations for RCU and DCU were above the cut-off value for MDMA, it is noteworthy that these concentrations are rather low and substantially lower than cocaine concentrations. Therefore, the present cocaine user samples had little poly-toxic drug use and did not differ from one another with regard to drugs other than cocaine.

Table 3. *Pattern and amount of self-reported drug use: Hair toxicology (means and standard deviations)*

	Stimulant-naïve controls (n=68)	**Recreational cocaine users (n=68)**	**Dependent cocaine users (n=30)**
Cocaine			
Cocaine (pg/mg)	5 (±20)	2740 (±4630)	19140 (±29170)
Benzoylecgonine (pg/mg)	0 (±5)	550 (±920)	4000 (±5735)
Ethylcocaine (pg/mg)	0 (±5)	280 (±320)	2040 (±3645)
Norcocaine (pg/mg)	0 (±1)	65 (±100)	490 (±590)
Amphetamine			
Amphetamine (pg/mg)	1 (±10)	75 (±260)	60 (±170)
Methamphetamine (pg/mg)	0 (±0)	1 (±10)	1 (±10)
Methylphenidate (pg/mg)	0 (±0)	10 (±55)	5 (±15)
MDMA			
MDMA (pg/mg)	1 (±15)	545 (±1600)	255 (±650)
MDEA (pg/mg)	0 (±0)	2 (±20)	0 (±0)
MDA (pg/mg)	0 (±1)	20 (±57)	10 (±30)
Opiates			
Morphine (pg/mg)	0 (±0)	3 (±25)	70 (±320)
Codeine (pg/mg)	0 (±2)	20 (±115)	35 (±115)
Methadone (pg/mg)	0 (±0)	1 (±10)	40 (±210)
EDDP (pg/mg)	0 (±0)	0 (±0)	5 (±25)
Tramadol	0 (±0)	3 (±17)	310 (±1640)

Pg/mg = picogram/milligram. The hair analysis was performed on two hair samples (each 3 cm in length) per participant capturing drug use over the last six months. Concentrations were averaged over the two samples. If the hair sample was not long enough, only one sample was analyzed (3 cm, 3 months). MDMA = 3,4-methylenedioxy-N-methylamphetamine; methylenedioxy-amphetamine, MDEA = methylenedioxyethylamphetamine, MDA = 3,4-methylenedioxyamphetamine, EDDP = primary methadone metabolite. Cut-off value for cocaine = 500 pg/mg, for amphetamines and MDMA = 200 pg/mg, for opiates = 200 pg/mg.

5.4.3 Domains of social and non-social decision-making

In a first step, we computed two domains of Social (SDM) and Nonsocial Decision-Making (NSDM) to obtain objective measures of how strongly cocaine users deviate from the performance of controls, to reduce the amount of data avoiding accumulation of the alpha-error. Lower scores in the SDM domain reflect a more selfish allocation of money in the games. Lower NSDM scores reflect inferior performance in the IGT and steeper discounting of delayed rewards (higher *k* parameters) in the Delay Discounting (DD), respectively. Overall, the SDM and NSDM domains did not correlate with one another (r=-.08). Moreover, neither the Distribution Game was associated with the IGT (r=-.01) and DD (r=-.11) nor was the Dictator Game (IGT, r=-.02; DD, r=-.05).

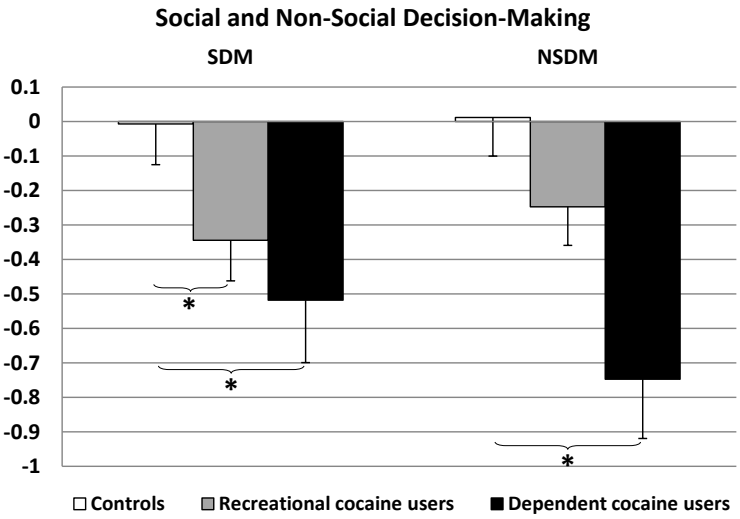

Fig. 1. Mean z-scores and standard errors for the social decision-making (SDM) and non-social decision-making (NSDM) domains. RCU and DCU deviate significantly from controls in SDM and DCU deviate significantly from controls in NSMD. Multiple regression analyses with age, sex, years of education, and dummy coded group contrasts (controls vs. RCU and controls vs. DCU): *p<.05.

Analysis of the SDM domain revealed that both RCU (β=-.17, t=-2.02, p<.05) and DCU (β=-.20, t=-2.35, p<.05) acted in a more selfish manner than controls. In the NSDM domain, DCU' performance was significantly inferior to controls (β=-.31, t=-3.67, p<.0001), while there was no significant difference between RCU and controls (**Fig. 1, Table 4, Table S2**).

5.4.4 Multiple regression analyses of substance use

Associations between drug use patterns and the SDM and NSDM domains were assessed by multiple regression models with cumulative drug use, weekly consumption, and duration of use as predictor variables. All three models had the common predictors of cocaine craving and positive cocaine urine toxicology to control for recent drug effects and craving urge (**Table S3**).

Table 4. *Behavioral task parameters (means, standard errors, and effect sizes)*

		Stimulant-naïve controls (n=68)	Recreational cocaine users (n=68)	Dependent cocaine users (n=30)	Cohen's *d* (Con/RCU)	Cohen's *d* (Con/DCU)
Z-Standardized Domain Scores						
Social decision-making		-0.01 (±0.12)	-0.34 (±0.12)	-0.52 (±0.18)	0.35*	0.45*
Non-social decision-making		0.01 (±0.11)	-0.25 (±0.11)	-0.75 (±0.17)	0.32	0.76*
Social Interaction Tasks						
Distribution Game	(payoff B)†	20.33 (±1.00)	17.82 (±1.00)	15.92 (±1.53)	0.31°	0.52*
Distribution Game	(total amount)	51.56 (±0.57)	50.14 (±0.57)	49.64 (±0.87)	0.31	0.36
Dictator Game	(payoff B)†	18.45 (±1.31)	15.02 (±1.31)	14.28 (±2.01)	0.30°	0.25°
Decision-Making Task						
Iowa Gambling Task	(total ratio; good - bad cards)§	18.63 (±3.14)	13.64 (±3.17)	7.91 (±4.83)	0.18	0.49°
Iowa Gambling Task	(total points)	4301.21 (±160.84)	4054.9 (±162.44)	3785.32 (±247.63)	0.18	0.41°
Delay Discounting	(*k* overall)§	0.013 (±0.004)	0.019 (±0.004)	0.034 (±0.006)	0.28	0.58**
	(*k* for large amounts)	0.009 (±0.003)	0.016 (±0.003)	0.031 (±0.005)	0.35	0.66**
	(*k* for medium amounts)	0.014 (±0.004)	0.020 (±0.004)	0.037 (±0.006)	0.27	0.64**
	(*k* for small amounts)	0.025 (±0.005)	0.039 (±0.006)	0.051 (±0.008)	0.34°	0.48**

All parameters are corrected for age, sex, and years of education. Con/RCU = controls vs. recreational cocaine users, Con/DCU = controls vs. dependent cocaine users.
†Used for the social decision-making composite score, §used for the non-social decision-making composite score.

Table S2. *Multiple regression analyses for demographic variables and group contrasts predicting task parameters*

	B	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
	Social Decision-Making			Non-Social Decision-Making			Distribution Game (payoff B)			Dictator Game (payoff B)		
Constant	-0.39	0.64		0.64	0.61		21.94	5.40		7.04	7.09	
Age	0.02	0.01	0.20*	-0.02	0.01	-0.16*	0.17	0.08	0.17*	0.26	0.1	0.20*
Sex	-0.07	0.17	-0.03	0.04	0.16	0.02	-1.36	1.40	-0.07	0.65	1.84	0.03
Years of education	-0.02	0.04	-0.04	-0.01	0.04	-0.03	-0.43	0.37	-0.09	0.23	0.48	0.04
Controls vs. RCU	-0.34	0.17	-0.17*	-0.26	0.16	-0.13	-2.51	1.41	-0.15°	-3.43	1.85	-0.16°
Controls vs. DCU	-0.51	0.22	-0.20*	-0.76	0.21	-0.31*	-4.41	1.84	-0.20*	-4.17	2.41	-0.15°
R^2		0.08			0.11			0.08			0.07	
F		2.91*			3.90**			2.70*			2.39*	

RCU = recreational cocaine users, DCU = dependent cocaine users. *p<.05, **p<.01, °p<.1.

Table S3. *Drug use patterns predicting social and non-social decision-making*

	SDM Model 1: Cumulative dose			SDM Model 2: Weekly consumption			SDM Model 3: Years of use			NSDM Model 1: Cumulative dose			NSDM Model 2: Weekly consumption			NSDM Model 3: Years of use		
	B	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*	*B*	*SE*	*β*
Constant	-0.15	0.53		-0.24	0.55		-0.33	0.35		0.53	0.50		-0.37	0.58		0.04	0.34	
Cocaine craving (CCQ)	-0.02	0.01	-0.22*	-0.02	0.01	-0.23*	-0.02	0.01	-0.17	-0.00	0.01	-0.01	-0.00	0.01	-0.03	-0.00	0.01	-0.03
Urine toxicology (pos./neg.)	-0.10	0.25	-0.04	-0.18	0.29	-0.09	-0.09	0.26	-0.04	0.13	0.24	0.05	0.03	0.30	0.01	0.14	0.25	0.05
Cocaine cum. dose (g)	0.21	0.16	0.14							-0.59	0.15	-0.37***						
Amphetamine cum. dose (g)	0.03	0.16	0.02							-0.11	0.15	-0.07						
MDMA cum. dose (pills)	-0.16	0.14	-0.13							0.18	0.13	0.14						
Cannabis cum. dose (g)	-0.09	0.08	-0.11							0.24	0.08	0.29**						
Cocaine (g/week)				0.30	0.39	0.12							-0.29	0.40	-0.09			
Amphetamine (g/week)				0.42	1.91	0.04							1.68	2.00	0.09			
MDMA (pills/week)				-0.01	0.95	-0.05							0.73	1.00	0.08			
Cannabis (g/week)				-0.14	0.39	-0.02							0.24	0.41	0.06			
Alcohol (g/week)				-0.00	0.21	-0.00							-0.05	0.22	-0.03			
Nicotine (cigarettes/week)				0.14	0.15	0.10							0.09	0.16	0.06			
Cocaine (years of use)							0.03	0.03	0.16							-0.09	0.03	-0.40**
Amphetamine (years of use)							0.01	0.04	0.03							0.01	0.04	0.04
MDMA (years of use)							-0.03	0.03	-0.10							-0.01	0.03	-0.04
Cannabis (years of use)							-0.01	0.02	-0.07							0.06	0.02	0.41**
Alcohol (years of use)							0.02	0.02	0.11							-0.00	0.02	-0.00
Nicotine (years of use)							0.00	0.02	0.02							-0.02	0.02	-0.16
R^2		0.09			0.06			0.10			0.22			0.03			0.21	
F		1.49			0.74			1.21			4.17**			0.37			2.86**	

N = 98. B = Unstandardized regression coefficient B, SE = Unstandardized standard error, β = Standardized Beta. Cum. dose = cumulative dose. *p<.05, **p<.01, ***p<.001. To avoid inflation of the results, only the cocaine user groups were analyzed.

None of the drug variables in the three models predicted behavior in SDM. In contrast, the cumulative dose of cocaine (β=-.37, t=-3.83, p<.001) and cannabis (β=.29, t=3.04, p<.01) predicted NSDM. Moreover, also duration of cocaine (β=-.40, t=-3.16, p<.01) and cannabis use (β=.41, t=3.39, p<.01) significantly explained NSDM variance. Interestingly, there was a negative relationship between NSDM and cumulative cocaine dose and duration of cocaine use, while the cumulative cannabis dose and duration of cannabis use were positively related to NSDM indicating that increased cocaine use was associated with worse NSDM, while more pronounced cannabis co-use correlated with better scores in NSDM. The putative positive effect of cannabis on NSDM is explained by lower discounting rates in the DD (cumulative life time dose r=-.35, p<.001; duration r=-25, p<.05), whereas cannabis use was not correlated with the IGT measures. Weekly consumption neither predicted SDM nor NSDM (**Table S3**).

5.4.5 Subanalyses of the social decision-making domain

The Distribution Game and Dictator Game allow measuring participants' social preferences in different tasks that tap into measures of fairness and efficiency. In the Distribution Game, DCU (β=-.20, t=-2.40, p<.05) and by trend also RCU (β=-.15, t=-1.78, p=.08) chose point distributions that were more profitable for themselves and yielded lower payoffs for participant B (**Table S2**). Overall, participants from all three groups chose the fair distribution most often. However, DCU chose the *unfair inefficient* distributions more frequently compared to controls (X^2(1)=10.74, p<.01) and RCU (X^2(1)=8.3, p<.01) (**Fig. S1 and S2**). Analyses regarding the Dictator Game showed trends that both RCU (β=-.16, t=-1.85, p=.07) and DCU (β=-.15, t=-1.73, p=.09) gave fewer points to their interaction partners compared to controls (**Table S2**).

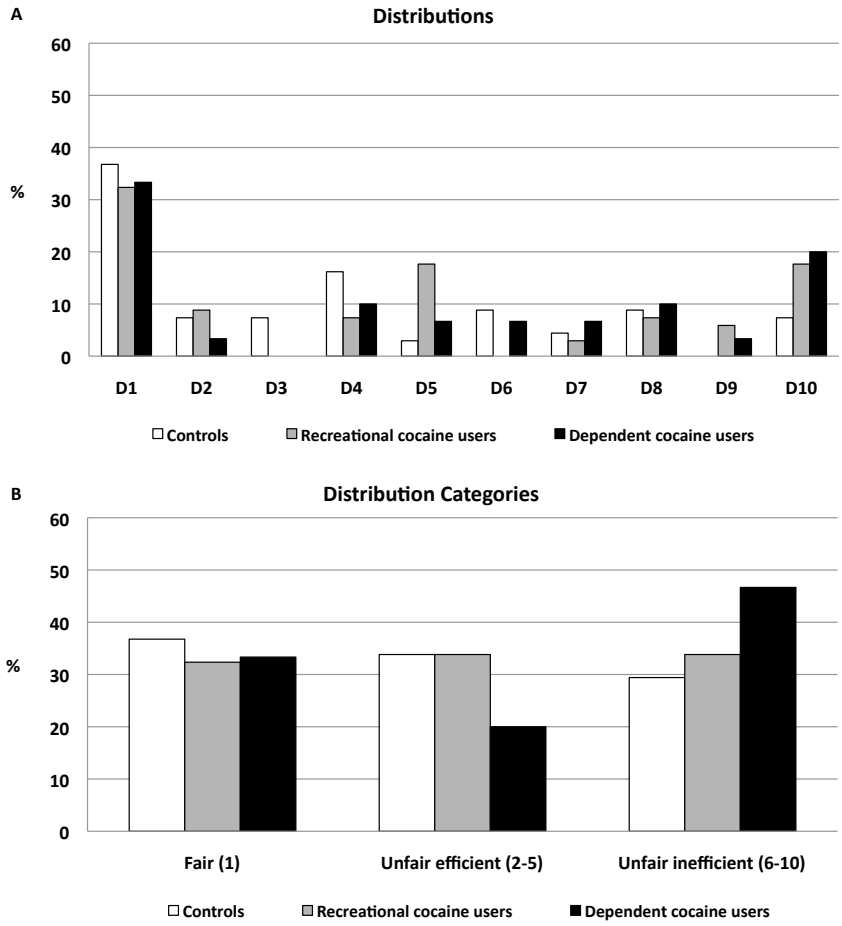

Fig. S2. *(A)* depicts the frequency in percent of the ten distributions of the Distribution Game chosen by controls, RCU and DCU. *(B)* displays the frequency in percent of the categories *fair*, *unfair efficient*, and *unfair inefficient* chosen by the three groups. DCU were less frequently classified as unfair efficient and more frequently as unfair inefficient.

Given that the Distribution and Dictator Game were carried out in succession, stability of fairness preferences can be assessed. Almost all participants remained *fair* in the Dictator Game if they had already been *fair* in the Distribution Game (**Fig. S3**). In contrast, controls who chose one of the distributions classified as *unfair efficient* in the Distribution Game, often chose a fair point allocation in the Dictator Game, while DCU who chose *unfair efficient* distributions in the Distribution Game were more likely to allocate points in the Dictator Game in a selfish manner ($X^2(1)=5.03$, $p<.05$). Furthermore, the number of subjects choosing one of the *unfair inefficient* distributions in the Distribution Game was substantially higher among RCU and DCU than among controls and both groups almost exclusively allocated points in an unfair manner in the Dictator Game ($X^2(1)=4.17$-5.03, $p<.05$).

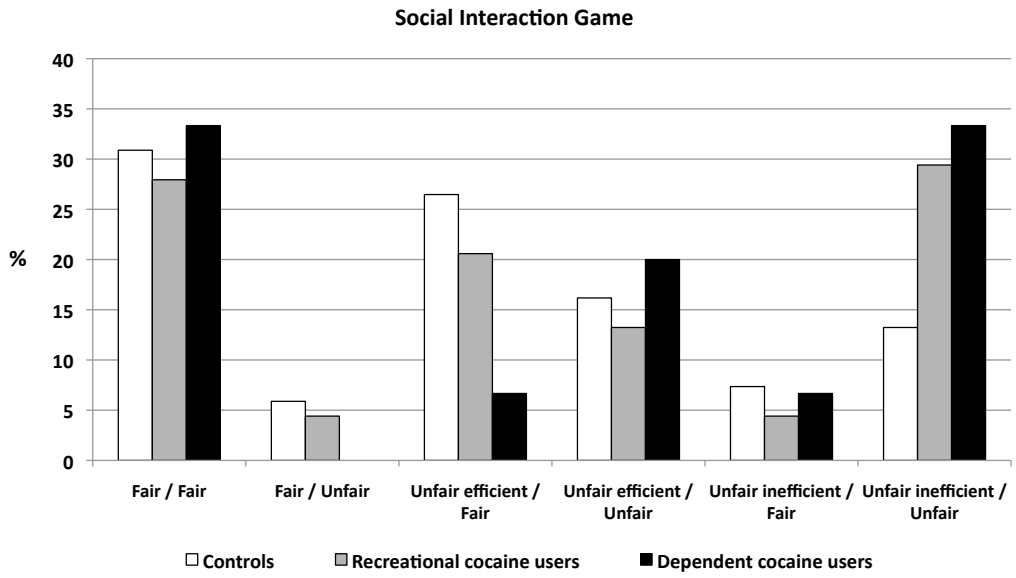

Fig. S3. The frequency in percent of how often the three groups chose the *fair*, *unfair efficient*, and *unfair inefficient* distributions in the Distribution Game are shown and whether they distributed the money in a fair or unfair manner in the Dictator Game. Almost all of the participants independent from which group they belong to, remained *fair* in the Dictator Game if they already had been *fair* in the Distribution Game. In contrast, controls who chose one of the distributions classified as *unfair efficient* in the Distribution Game, often chose a fair point allocation in the Dictator Game, while DCU who also chose one of the distributions classified as *unfair efficient* in the Distribution Game were more likely to allocate points in the Dictator Game in a selfish manner. Moreover, the number of subjects choosing one of the *unfair inefficient* distributions in the Distribution Game was substantially higher among RCU and DCU than among controls, and these RCU and DCU almost exclusively allocated the money in an unfair manner in the Dictator Game.

5.4.6 Sub-analyses of the non-social decision-making domain

A repeated-measures ANCOVA revealed that in the IGT, despite the fact that overall, both RCU (net score: d=.18) and particularly DCU (d=.49) chose fewer favorable cards than controls, no statistically significant group effect emerged ($F(2,159)=1.81$, $p=.17$). As expected, the factor quartile was significant ($F(3, 477)=2.83$, $p<.05$), suggesting a learning curve (**Fig. S4*A***). Correlation analyses indicated that the performance of the IGT was negatively related to the duration of cocaine use ($r=-.35$, $p<.001$) and the cumulative cocaine dose ($r=-.25$, $p<.05$).

In the DD, groups significantly differed in their preferences for smaller immediately and larger delayed monetary rewards ($F(2, 163)=6.52$, $p<.01$; **Fig. S4*B***). Sidak-corrected *post hoc* comparisons showed that DCU were more likely to choose immediate rewards compared to controls ($p<.01$). As expected, discounting of delayed rewards varied with reward magnitude ($F(2, 326)=34.79$, $p<.001$). Correlation analyses showed that the overall k ($r=.23$, $p<.05$), and k for medium ($r=.23$, $p<.05$) and

small amounts (r=.20, p<.05) correlated with the cumulative cocaine dose. Interestingly, k for medium amounts was strongly related to the cocaine metabolite ethylcocaine (r=.37, p<.0001), indicating that especially subjects who consumed cocaine in combination with alcohol showed increased levels of impulsivity with regard to reward (Pennings et al., 2002).

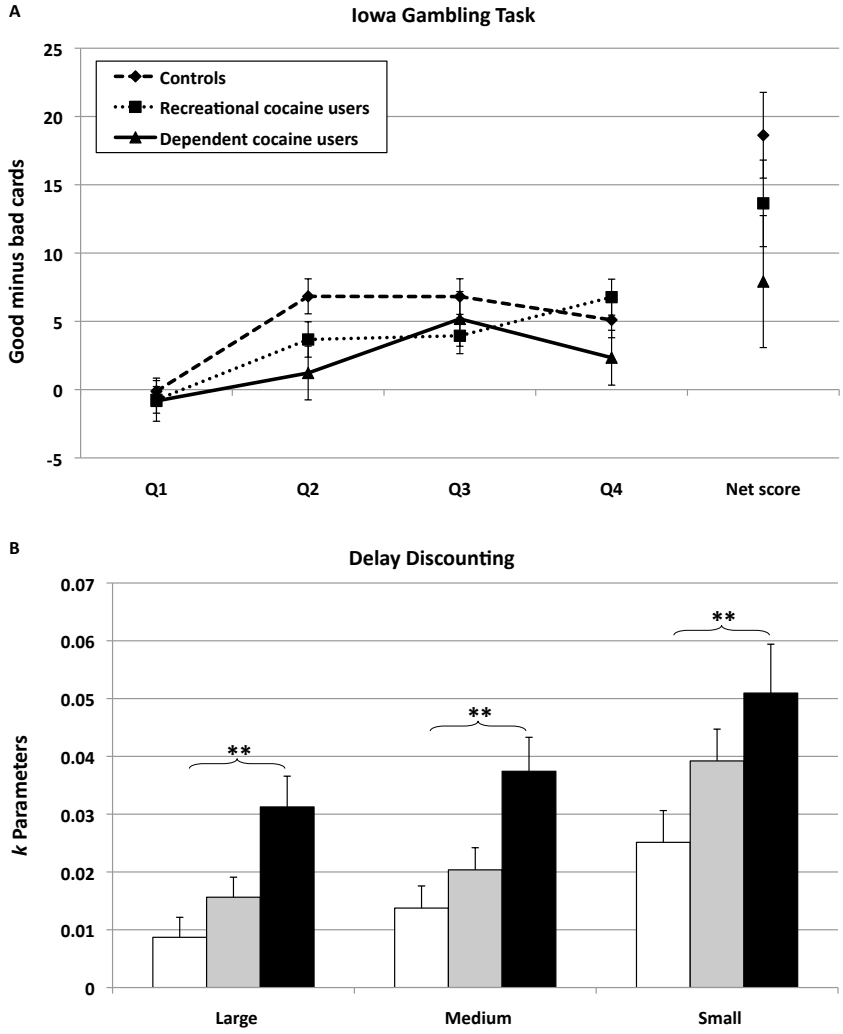

Fig. S4. *(A)* Means and standard errors for quartiles and the net score (advantageous minus disadvantageous cards) in the Iowa Gambling Task. Particularly DCU exhibited a decreased learning curve and chose more unfavorable cards than controls. *(B)* Means and standard errors for large, medium, and small k parameters. DCU discounted delayed rewards more steeply than controls. Sidak-corrected post hoc analyses: **p<.01.

Additional analyses were conducted to investigate potential effects of psychiatric symptoms, cocaine craving, and recent drug use on SDM and NSDM (**Supplement Text**, **Table S4**, **Fig. S5**). Both, cocaine users with and without clinically relevant ADHD symptoms exhibited lower SDM and NSDM scores than controls. Therefore, presence of ADHD symptoms alone cannot explain our

results. Importantly, mainly cocaine users with high cocaine craving scores, acted in a more selfish manner in the SDM tasks compared to controls. In contrast, craving did not impact NSDM. Recent cocaine intake did not significantly impact SDM and NSDM scores. Moreover, primarily cocaine users with slightly elevated symptoms of depression (BDI scores >10) performed significantly worse than controls with low BDI scores in NSDM, while symptoms of depression did not influence SDM of cocaine users.

Table S4. *Multiple regression analyses for demographic variables and group contrasts predicting task parameters*

	Social Decision-Making			Non-Social Decision-Making		
Constant	-0.42	0.64		0.55	0.62	
Age	0.02	0.01	0.19*	-0.02	0.01	-0.19*
Sex	-0.06	0.17	-0.03	0.05	0.16	0.02
Years of education	-0.02	0.04	-0.03	0.00	0.04	0.01
Controls vs. CU without ADHD	-0.36	0.16	-0.18*	-0.39	0.16	-0.20*
Controls vs. CU with ADHD	-0.50	0.24	-0.17*	-0.49	0.23	-0.17*
R^2		0.08			0.08	
F		2.86*			2.69*	
Constant	-0.44	0.63		0.55	0.62	
Age	0.02	0.01	0.19*	-0.02	0.01	-0.19*
Sex	-0.08	0.16	-0.04	0.05	0.16	0.02
Years of education	-0.01	0.04	-0.02	0.00	0.04	0.01
Controls vs. CU with low craving	-0.22	0.18	-0.10	-0.42	0.17	-0.20*
Controls vs. CU with high craving	-0.57	0.18	-0.26**	-0.40	0.18	-0.19*
R^2		0.10			0.08	
F		3.49**			2.65*	
Constant	-0.41	0.64		0.54	0.62	
Age	0.02	0.01	0.19*	-0.02	0.01	-0.19*
Sex	-0.12	0.17	-0.05	0.05	0.16	0.02
Years of education	-0.01	0.04	-0.02	0.01	0.04	0.01
Controls vs. CU with neg. cocaine UT	-0.33	0.16	-0.17*	-0.41	0.16	-0.21*
Controls vs. CU with neg. cocaine UT	-0.47	0.24	-0.17*	-0.38	0.23	-0.14°
R^2		0.08			0.08	
F		2.82*			2.61*	
Constant	-0.50	0.65		0.62	0.60	
Age	0.02	0.01	0.20*	-0.02	0.01	-0.16*
Sex	-0.09	0.17	-0.04	-0.04	0.16	-0.02
Years of education	-0.01	0.04	-0.01	0.01	0.04	0.01
Controls with low BDI vs. CU with low BDI	-0.34	0.17	-0.17*	-0.23	0.16	-0.12
Controls with low BDI vs. CU with high BDI	-0.48	0.22	-0.19*	-0.94	0.20	-0.38**
R^2		0.08			0.15	
F		2.65*			5.42**	

CU = cocaine users, UT = Urine toxicology, BDI = Beck depression inventory (low BDI < 11). *p<.05, **p<.01, °p<.1.

5.5 Discussion

In this study, we report on differences in individual and social decision making in RCU and DCU in comparison to a psychostimulant-naïve control group. Careful psychiatric diagnostic procedures ensured that cocaine users had few psychiatric co-morbidities and detailed hair toxicology analyses showed relatively sparse poly-substance use. Our study yielded the following major findings: I) during social interaction, both RCU and DCU distributed money in a more self-opportune manner than controls. More specifically, both groups took more money for themselves in the Distribution Game and gave less money to the second interaction partner in the Dictator Game. II) DCU but not RCU exhibited lower NSDM scores than controls, reflecting that DCU chose fewer advantageous cards in the IGT and showed elevated reward-related impulsivity in the DD compared to controls. Higher cumulative doses of cocaine and duration of use were associated with lower NSDM scores but not with SDM scores. Moreover, ADHD symptoms and recent drug use were not related to the performance in SDM and NSDM in cocaine users. Interestingly, craving seemed to influence SDM preferences as cocaine users with high but not with low cocaine craving scores acted in a more selfish manner in the money distribution games than controls. Symptoms of depression had an additional impact on NSDM but not on SDM. Taken together, our results indicate that both RCU and DCU show deficits in SDM whereas only DCU are impaired in NSDM.

To our knowledge, no studies have assessed human social interaction using an experimental economic approach in cocaine addiction research so far. We observed that control subjects who chose one of the distributions classified as *unfair efficient* in the Distribution Game, often chose a fair point allocation in the Dictator Game, while DCU who also chose one of the distributions classified as *unfair efficient* in the Distribution Game were more likely to allocate points in the Dictator Game in a selfish manner. Thus, it appears that the proportion of controls who seem to place a higher value in efficient distributions do this at the cost of fairness towards the other player. However, if efficiency preferences do not matter, as in the Dictator Game, the same subjects still care for fairness. This is not observed in those DCU who chose efficient distributions in the Distribution Game, as they seem to care only about efficiency and less about fairness. Furthermore, among RCU and DCU the number of subjects choosing one of the *unfair inefficient* distributions in the Distribution Game was substantially higher and among these, almost everyone allocated points in an unfair manner in the Dictator Game. Overall, these findings suggest that cocaine users are less concerned about fairness in dyadic interactions, compared to controls. Although the self-serving behavior was more pronounced in DCU than RCU when compared to controls, the absence of a significant correlation between amount of cocaine use and selfishness could signify that cocaine users may have a *predisposition* towards more

self-serving behavior. Additionally, also cocaine craving enhances the propensity to act selfishly, indicating that SDM preferences in cocaine users have also a state component.

Because of the cross-sectional design of our study, it is impossible to substantiate whether the differences in SDM among cocaine users and controls are due to a certain predisposition, drug-induced cerebral alterations, or an interaction thereof. As a prior study showed that chronic cocaine use is associated with selective deficits with regard to higher-level emotional reasoning such as understanding, managing, and regulating emotions (Fox et al., 2011), our results on social interaction can be understood in the way that cocaine users might have personality traits that hinder them to adopt another person's perspective and to feel empathy or guilt. Alternatively, converging findings suggest that the ventromedial PFC (VMPFC), a brain region that has been shown to be altered in cocaine users (Goldstein and Volkow, 2011), is a critical node in regulating social interaction. For instance, studies with patients featuring lesions in the VMPFC have given account of marked social impairment in the form of inappropriate social behavior and poor decision-making judgment (Damasio, 1994). Such patients also gave significantly less in money distribution (Krajbich et al., 2009) and showed an abnormally utilitarian pattern of judgments on moral dilemmas (Koenigs et al., 2007). A further brain region that is critically involved in SDM and has been shown to be altered in cocaine users is the DLPFC (Goldstein and Volkow, 2002). The DLPFC is of crucial importance for exerting cognitive control or self-control (Hare et al., 2009; Miller and Cohen, 2001) as well as for bringing about norm-related behavior and thereby overriding selfish behavioral impulse (Sanfey et al., 2003; Spitzer et al., 2007). Therefore, cocaine-related neuroadaptations in the VMPFC may decrease the ability to feel empathetic towards interaction partners and alterations in the DLPFC might reduce the inability to control selfish urges in cocaine users, particularly during strong cocaine craving.

Regarding NSDM, the results of the present study were largely consistent with earlier data (Bechara et al., 2002; Kjome et al., 2010; Verdejo-Garcia et al., 2007*a*). Although RCU and in particular DCU chose fewer advantageous cards in the IGT than controls, the differences were not statistically significant, which is consistent with results from a prior study (Bolla et al., 2003) but not others (Bechara et al., 2002; Kjome et al., 2010; Verdejo-Garcia et al., 2007*a*). The lack of statistical significance in our study might be explained by two reasons: I) We applied stringent criteria to exclude subjects with severe psychiatric co-morbidities and toxicological hair analyses ensured that participants had little co-use of other illegal drugs. Therefore, even the DCU of the present study might have a higher level of general functioning compared to those of other study samples (Bechara et al., 2002; Kjome et al., 2010; Verdejo-Garcia et al., 2007*a*, *b*). II) The smaller effect sizes in the IGT in our study might be explained by the fact that the IGT gains were paid, which is in line with observations of a previous study reporting impaired IGT performance in cocaine users only if monetary gains were hypothetical but not when the money they won was actually paid (Vadhan et al., 2009).

Here we replicate previous results on intertemporal choice in DCU (Bickel et al., 2011*a*; Heil et al., 2006; Kirby and Petry, 2004). Importantly, we found that even RCU exhibited steeper discounting rates than controls with small to moderate effect sizes across reward magnitudes (d=.27-.35). Thus, even predisposed tendencies of impulsive decision-making may render RCU more prone to initiate drug use, and subsequently, neuroadaptations induced by repeated cocaine use may amplify preexisting reward impulsivity resulting in the well-described compulsive drug-seeking behavior – the inability to forego rewarding short-term effects of the drug in favor of the long-term benefits associated with abstinence (Bolla et al., 1998). In fact, DD has been shown to have trait-like stability (Casey et al., 2011; Mischel et al., 2011; Odum, 2011) and a prospective study revealed that the ability to delay gratification in childhood predicted physical health, substance dependence, finances, and criminal offences in adulthood {Moffitt, 2011 #98}. On the other hand, animal studies suggest that chronic administration of cocaine can cause sustained elevations in impulsive choice in rats and monkeys (Mendez et al., 2010; Olausson et al., 2007), indicating that decision-making deficits in cocaine users could also be drug-induced. A brain area that may be particularly important for intertemporal discounting is the DLPFC because transient disruption of the left DLPFC with repetitive transcranial magnetic stimulation increased impulsive choice behavior in healthy subjects (Figner et al., 2010). Therefore, steeper discounting functions in cocaine users may be associated with neuroadaptations and dysfunction of the DLPFC (Goldstein and Volkow, 2002). As decision-making performance in the IGT and the DD was correlated with drug use parameters, this suggests indeed that cocaine-induced neuroadaptations may contribute to decision-making deficits.

Because psychiatric co-morbidities such as ADHD are frequently present among addicted individuals (Ivanov et al., 2008; Perez de Los Cobos et al., 2011), we investigated how ADHD symptoms influence decision-making behavior. Cocaine users showed impaired SDM and NSDM scores compared to controls irrespective of whether they scored high or low on ADHD symptoms. Therefore, ADHD symptoms did not seem to have an impact on decision-making performance in the current study. Interestingly, cocaine users with high but not those with low levels of cocaine craving acted more selfishly compared to controls in SDM, while craving intensity was not related to NSDM. One could speculate that strong craving urges may have fostered thoughts about obtaining cocaine as soon as possible, which could have led cocaine users to maximize their monetary profit. Recent drug use did not seem to influence decision-making as cocaine users had lower SDM and NSDM scores irrespective of whether urine toxicology was positive or negative for cocaine. Especially cocaine users with slightly elevated depression scores performed significantly worse than controls with low depression scores in NSDM, while symptoms of depression did not seem to impact on SDM.

The current findings should be interpreted bearing some limitations in mind. Given the cross-sectional design it is not possible to conclusively answer whether deficits in SDM and NSDM precede cocaine use or are due to cocaine-induced neuroadaptations. Therefore, data from longitudinal and

prospective investigations are desirable to further decompose the effects of predisposition and sequelae of cocaine use. In the current study, we merely obtained behavioral results. Combining functional imaging with behavioral measures could be of great importance for future studies. Finally, in order to guarantee anonymity of the cocaine users, we had to use a cover story in the social interaction paradigms. However, we assessed whether participants had doubts about a real interaction and introduced this measure as a covariate into the statistical analyses, which did not change the results.

Identifying vulnerability markers and adverse drug-induced effects with regard to impaired decision-making in cocaine users is critical and may be of great benefit for the development of successful prevention and treatment strategies enhancing quality of life substantially. For example, it was recently demonstrated that working memory training decreased the propensity to discount delayed rewards in stimulant addicts (Bickel et al., 2011). Likewise, knowledge from tasks measuring SDM could be integrated in therapeutic interventions for cocaine-addicted individuals for example in the form of social skills trainings.

5.5.1 Conclusion

In sum, these findings are the first to show that RCU and DCU both exhibit more opportunistic behavior regarding money allocation in social interaction paradigms. Interestingly, mainly the DCU performed worse in the IGT and showed elevated reward-related impulsivity compared to controls. Considering the intermediate decision-making performance of RCU compared to controls and DCU as well as the association of the worse decision-making performance in the IGT and the steeper discounting rates with cumulative cocaine use and duration of cocaine use, decision-making deficits in cocaine users may be due to an interaction of predisposing and drug-induced effects. The sample of the present study is unique due to careful psychiatric diagnostic procedures and objective hair toxicology analyses that ensured that cocaine users had modest psychiatric co-morbidity and little poly-substance use. Our results have implications for the conceptualization of treatment approaches that specifically target social interaction and decision-making deficits in cocaine users. For instance interventions in the form of social skills training or cognitive remediation strategies may be especially beneficial for improved quality of life and successful abstinence.

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5.7 Supplemental information

5.7.1 Methods

Participants

Subjects were recruited in Zurich via advertisements in widely read local newspapers, different drug prevention and treatment centers, psychiatric hospitals, Internet platforms, and word-of-mouth communication. The recruiting period took place between January 2010 and January 2012. Eight-hundred-four prospective participants completed an initial telephone screening, whereof 240 subjects participated in the actual study. Forty-six participants had to be excluded afterwards because hair analyses revealed illegal drug use not declared in the interviews (e.g., opioids, excessive MDMA use) or lack of cocaine use. Twenty-eight participants (18 controls, 10 cocaine users) were excluded to achieve adequate matching across the three groups (i.e., controls with particularly high verbal IQ, older dependent cocaine users [DCU]), very young recreational cocaine users [RCU]), resulting in a final sample size of 166 participants.

Study procedure

Participants completed a large test battery comprising clinical interviews and questionnaires, neuropsychological, electrophysiological, and social and non-social decision-making measures. The time used to complete the test battery ranged from 4 to 6 hours with breaks after every 1.5 h. Participants were allowed to take additional breaks as needed and smoking was permitted ad libitum. The longitudinal data and results from neuropsychological (Vonmoos et al., 2012) and psychophysiological assessments will be published elsewhere (Preller et al., 2012).

Behavioral tasks

Social decision-making: In the social interaction tasks, participants were told that they would interact with two other study participants in a randomly assigned role of either player A or B. In order to warrant anonymity of the drug users, which was necessary for ethical reasons and for professional secrecy, a cover story was used where players were told that they would interact with the other subjects via Internet connection. For the purpose of this study, we were solely interested in the role of player A, wherefore player B was simulated by the computer and always responded in the same manner. The instructions specify that both players are informed about the other player's possibilities of action and that the points will be converted into real money (Swiss Francs, CHF) at the end of the study. The plausibility of the cover story was controlled by the following question at the end of the test battery: "For reasons of anonymity you did not meet your interaction partner personally. Did you

have any doubts that you interacted with someone?" Subjects responded on a five-point scale ranging from *not at all* to *very much*.

Non-Social Decision-Making: The IGT tests the ability to choose between favorable card decks with lower gains but also a lower risk for losses eventually resulting in long-term benefit and unfavorable card decks with higher gains but also higher losses resulting in long-term loss. In this study, a computerized version of Grasman and Wagenmakers (University of Amsterdam, Netherlands) was used (http://purl.oclc.org/NET/rgrasman/jscript/IowaGamblingTask). In total, participants had to draw 100 cards, whereby each card deck contained 50 cards. Dependent variables were the net score of favorable and unfavorable cards drawn, the total number of points gained, and quartiles (the 100 cards were summed in 4 quartiles each containing 25 cards). At the end of the task, points were converted by the factor .002 and paid out in real money. All participants started out with 4000 points, with the maximum number of points that could be gained at 8000, equaling 16 CHF.

In the DD, subjects were presented with a choice between an immediately available lower monetary reward and a higher reward available with a temporal delay. The discounting rate can be calculated with the Formula $V=A/(1+ kD)$(V is the present value of the delayed reward A at delay D, and k is a free parameter that determines the discount rate) (Mazur, 1987). In this study, we used a computerized version (implemented in Presentation®) of the DD paradigm according to Kirby et al. (1999). This version not only makes it possible to investigate the steepness of discounting of delayed rewards (expressed as k; the larger the parameter k, the stronger the discounting of larger delayed rewards), but also distinguishes between large, medium, and small rewards.

Methodology of the urine analysis

Urine toxicology analyses comprised the compounds/substances tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and were assessed by a semi-quantitative Enzyme Multiplied Immunoassay method (Dimension RXL Max, Siemens, Erlangen, Germany) (SAMHSA, 2008).

Methodology of the hair analysis

If participants' hair was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. Hair samples from 163 subjects were successfully analyzed. Due to an insufficient amount of hair, the samples from two controls, and one cocaine user could not be analyzed properly. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine, MDMA, MDEA, MDA, morphine, codeine, methadone EDDP (primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis a three step washing procedure with water (2 minutes shaking, 15ml), acetone (2min., 10ml) and finally hexane (2min., 10ml) of hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5ml, 16hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33 % (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5. MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of HPLC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge, (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1mM ammonium formate buffer adjusted to pH 3,5 with formic acid (eluent A) and acetonitrile containing 1mM ammonium formate and 1 mM formic acid (eluent B). The Analysis was performed in MRM mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

5.7.2 Results

Illegal drug use

DCU smoked significantly more cigarettes ($F(2, 163)=4.59$, $p<.05$) and consumed more alcohol ($F(2, 163)=4.28$, $p<.05$) per week compared to controls. Both RCU and DCU had a significantly longer duration of cannabis use and RCU had a higher cumulative dose of hallucinogens in comparison to controls. DCU had a higher cumulative dose of cannabis in comparison to controls and RCU. Moreover, DCU reported to use cocaine more frequently, for a longer duration than RCU, and had higher cocaine and 3,4-Methylendioxy-N-methylamphetamin (MDMA) cumulative doses than RCU, while amphetamine and 4-hydroxybutanoic acid (GHB) consumption was comparable among

the cocaine user groups. Stimulant-naïve controls were allowed to have recreational cannabis use and limited (<15 times) experiences with other illegal drugs.

Subanalyses of potential co-factors

Because both RCU and DCU scored substantially higher in a questionnaire assessing symptoms of ADHD and depression we sought to investigate how strongly these symptoms impact SDM and NSDM. In addition, as strong cocaine craving and acute drug intoxication are likely to influence task performance, we examined whether the degree of craving and whether participants tested positive for cocaine in the urine toxicology influenced the decision-making performance (**Fig. S5**). For these analyses the two cocaine users groups were merged. As summarized in **Table S4**, multiple regression analyses were calculated with the common variables of age, sex, and years of education included in the model and dummy coded group variables. The first model addressed whether clinically relevant ADHD symptoms were related to the decision-making performance in cocaine users. Both cocaine users without (n=76, β=-.18, t=-2.20, p<.05) and with clinically relevant ADHD symptoms (n=22, β=-.17, t=-2.10, p<.05) exhibited more selfish SDM and inferior NSDM (β=-.20, t=-2.46, p<.05; β=-.17, t=-2.09, p<.05) compared to controls without diagnosed ADHD. Therefore, ADHD symptoms alone cannot be responsible for impaired performance as also cocaine users without ADHD showed deficits in SDM and NSDM.

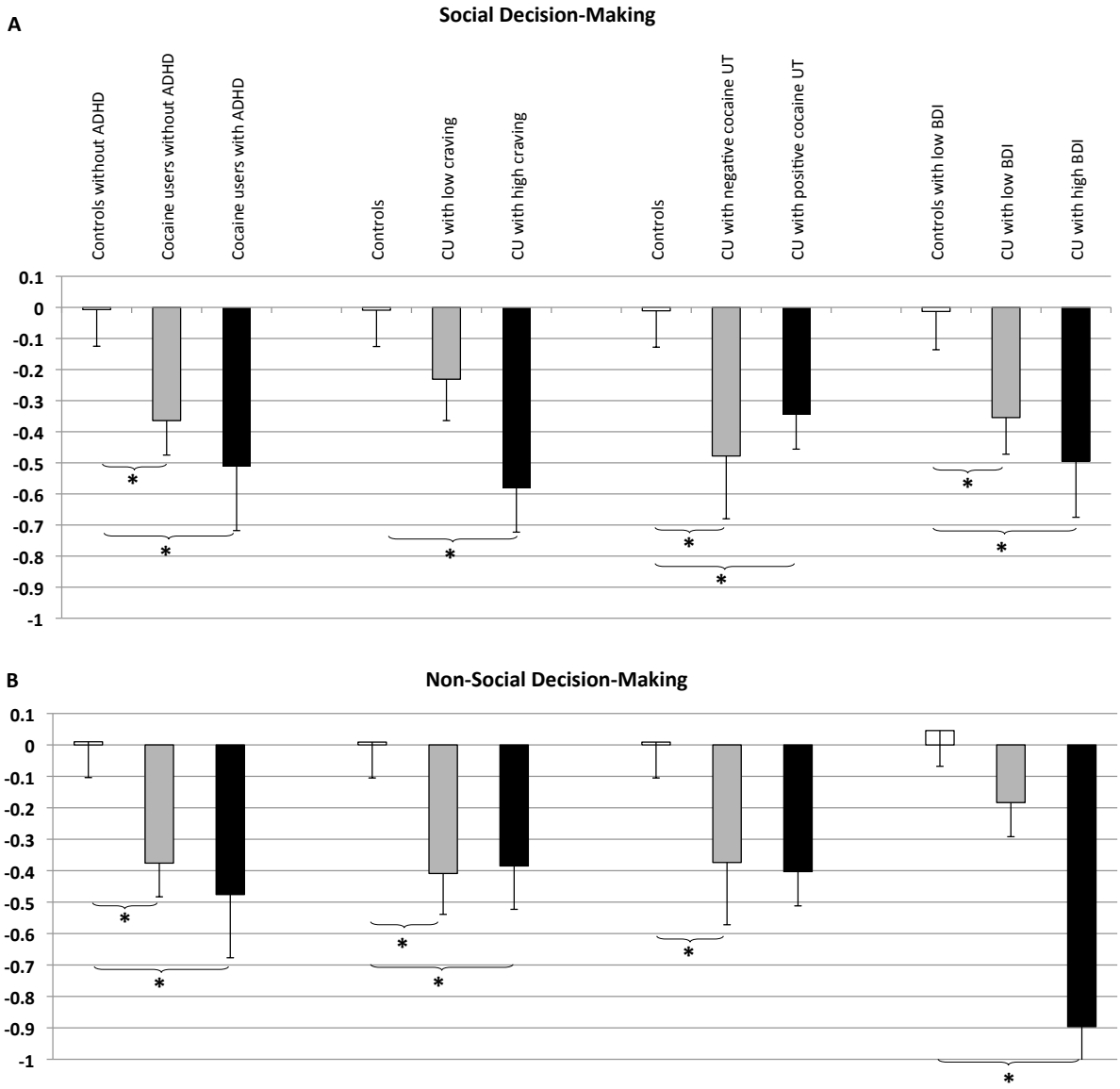

Fig. S5. Mean scores and standard errors subdivided in cocaine user groups without and with clinically significant ADHD symptoms, low and high cocaine craving, negative and positive cocaine urine toxicology (UT), and low and high depression scores (BDI). *(A)* the social decision-making domain (SDM) and *(B)* the non-social decision-making domain (NSDM). Multiple regression analyses with age, sex, years of education, and dummy coded group contrasts (controls vs. RCU, controls vs. DCU): **p*<.05, ***p*<.01.

Cocaine users with high cocaine craving scores (n=46, *ß*=-.26, *t*=-3.13, *p*<.01) but not cocaine users with low craving scores (n=52), acted in a more selfish manner in the SDM tasks compared to controls. In contrast, NSDM was impaired in cocaine users with low (*ß*=-.20, *t*=-2.41, *p*<.05) and high craving scores (*ß*=-.19, *t*=-2.20, *p*<.05). Consequently, craving seemed to have a stronger impact on social interaction than on non-social decision-making processes.

Both cocaine users who tested negative (n=74, β=-.17, t=-2.09, p<.05) and cocaine users who tested positive (n=23, β=-.17, t=-2.02, p<.05) for cocaine in the urine toxicology exhibited more selfish behavior in the SDM tasks than controls. With regard to NSDM, both cocaine users who tested negative for cocaine (β=-.21, t=-2.60, p<.05) and by trend also cocaine users with a positive cocaine urine toxicology (β=-.14, t=-1.68, p=.096) had lower scores than controls. Thus, recent drug use is not responsible for the impaired SDM and NSDM.

Cocaine users with low (BDI score < 11) (n=69, β=-.17, t=-1.99, p<.05) and high depression scores (n=29, β=-.19, t=-2.20, p<.05) acted more selfishly in the SDM tasks, indicating that symptoms of depression did not have an impact on SDM. Higher depression scores were associated with lower NSDM scores, as reflected by the fact that only cocaine users with high (β=-.38, t=-4.67, p<.001) but not low depression scores performed significantly worse in NSDM compared to controls with low depression scores.

5.7.3 References

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6

GENERAL DISCUSSION

Despite the growing understanding about molecular mechanisms underlying the transition from regulated to uncontrolled, addicted cocaine use and associated behavioural deficits, therapeutic interventions have limited success as evidenced by the frequent relapses occurring in dependent cocaine users. Given that no effective medications are currently available, cocaine addiction continues to be a major challenge for society, economy, and its treatment. The present thesis attempted to contribute to bridging lacking knowledge in three areas: The first study concerned itself with how cocaine use is associated with colour vision impairment and cognitive deficits, which has been hypothesized to be mediated by altered retinal and cerebral dopaminergic neurotransmission that might correspond to some extent. The second study investigated if the density of mGluR5s differs between cocaine users and drug-naïve controls. The mGluR5 is of interest due to its predominant localization in addiction-related corticolimbic brain regions and its implication in reinstatement of cocaine-seeking behaviour in preclinical studies. The third study examined if even recreational cocaine users who do not meet cocaine dependence criteria exhibit decision-making deficits and whether these deficits extend to social decision-making as well. In this final section, findings from the three empirical studies of the present thesis shall be briefly summarized. Subsequently, strengths and limitations of the studies will be presented, and finally, implications, unresolved issues, and future directions shall be discussed.

6.1 Blue-yellow colour vision impairment in cocaine users

Our findings confirmed our hypotheses raised for the first study by revealing that both recreational and dependent cocaine users exhibited more frequent and more intense colour vision impairment, predominantly in the blue-yellow spectrum, in comparison to psychostimulant-naïve controls. Moreover, only MDMA users with strong exposure to amphetamine showed colour vision impairment comparable to cocaine users, whereas pure MDMA users did not display colour vision impairment. Higher colour confusion indices were associated with higher cumulative use of cocaine and longer duration of use. In addition, verbal declarative memory performance was inferior in cocaine users with colour vision disorder compared to users and controls with intact colour vision and worse colour discrimination indices and cognitive deficits both correlated with higher cumulative cocaine use and longer duration of cocaine use. To what extent the colour vision impairment observed in stimulant users may reflect altered retinal and central dopamine neurotransmission cannot be answered by our study design as the LD-15 colour vision discrimination test is not a direct marker of dopamine function. However, the specific impairment associated with the use of amphetamine and cocaine but not MDMA as well as the relationship between colour vision disorders and cocaine use parameters

and cognitive deficits may provide some support for this notion. However, future research should objectively test these hypotheses, which will be discussed further in section 6.6.

Altogether, these findings are consistent with prior studies revealing specific blue-yellow colour vision impairment in dependent cocaine users (Desai et al., 1997, Roy et al. 1996, 1997a, 1197b, 2003). We extended previous findings by showing that even non-dependent, recreational cocaine users exhibit diminished blue-yellow colour vision discrimination and that colour vision impairment is specific for drugs mainly altering the dopamine system such as cocaine and amphetamine but not for drugs primarily affecting serotonergic neurotransmission such as MDMA. In addition, we were the first to show that colour vision impairment is associated with cognitive deficits in cocaine users, possibly reflecting a potential relationship between retinal and cerebral dopaminergic alterations.

6.2 mGluR5 availability in human cocaine users

In the second study of the present thesis, we were the first to investigate mGluR5 availability in human cocaine users. Our results revealed that mGluR5 density in cocaine users, measured by means of the highly selective mGluR5 antagonist ^{11}C-ABP688, did not differ from drug-naïve controls. Furthermore, frequency, amount, and duration of cocaine use did not correlate with mGluR5 availability in cocaine users. Contrariwise, smoking was associated with a marked global decrease with an average of 20% in mGluR5 availability in all predefined brain regions of interest, independent of cocaine use. Shorter nicotine abstinence was associated with lower mGluR5 density, indicating that the decrease in mGluR5 density was particularly pronounced in individuals who had smoked very recently. Although our initial hypotheses, derived on the basis of preclinical studies, were not confirmed, these findings contribute further to the understanding of which particular aspects in the glutamate system may or may not become altered through cocaine use. However, it is noteworthy that we cannot fully exclude the possibility that mGluR5 availability in the NAcc core may be reduced in cocaine users compared to controls. Due to spatial limitations inherent to PET analyses we could not investigate this sub-region of the NAcc (see section 6.4). However, we will conduct additional analyses where GluR5 availability in the whole NAcc region will be quantified. Moreover, the present study cannot ascertain if functional alterations of mGluR5s may have occurred in cocaine users. Our findings highlight the need for further studies aimed at establishing a more complete understanding regarding which molecular alterations may occur in the glutamate system of human cocaine users. Therefore, we measured free glutamate levels in the postgenual anterior cingulate gyrus and the DLPFC by means of MRS and will examine whether free glutamate levels correspond to mGluR5 availability in these regions.

Overall, these findings have important implications for the development of novel pharmacotherapies aimed at facilitating smoking cessation. The observed down-regulation of mGluR5s in smokers may constitute a compensatory, protective mechanism to prevent overstimulation of mGluR5s akin to observed down-regulation of mGuR5s in cocaine-addiction models in rats (Kalivas, 2009). More research is required to clarify if mGluR5 antagonists in human cocaine and nicotine users may have a similarly beneficial effect to prevent relapse as has been observed in preclinical studies where blocking mGluR5s attenuated reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin seeking (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Paterson and Markou, 2005; Paterson et al. 2003; Platt et al., 2008; van der Kam et al. 2007).

6.3 Non-social and social decision-making in cocaine users

The third study of the present thesis was the first to assess social interaction behaviour in cocaine users by means of an experimental economic approach. The key finding was that recreational and dependent cocaine users distributed money in a more self-serving manner than controls. More specifically, both cocaine user groups took more money for themselves in the Distribution Game and gave less money to the second interaction partner in the Dictator Game. In contrast, only dependent cocaine users performed worse in non-social decision making tasks reflecting that they chose fewer advantageous cards in the IGT and showed elevated reward-related impulsivity in the DD compared to controls. Interestingly, cocaine use patterns were associated with non-social but not social-decision making, possibly implying that deficits in non-social decision-making could be partially induced by cocaine use, whereas predisposing personality traits may predominantly account for the more self-serving choices during social interaction in cocaine users. Further noteworthy, craving but not socioeconomic status was associated with social decision-making preferences as cocaine users with high but not with low cocaine craving scores acted in a more selfish manner in the money distribution games compared to controls. Accordingly, an interaction of trait and state measures seems probably in social decision-making. However, to substantiate the matter of causality, longitudinal and prospective study designs will be required.

Combined, our results suggest that both recreational and dependent cocaine users exhibit deficits in social decision-making, whereas only dependent cocaine users show compromised non-social decision-making.

6.4 Strengths and limitations of the present studies

The three experimental studies conducted in the present thesis feature a number of strengths meriting discussion. First of all, including a group of recreational cocaine users who do not meet the DSM-IV criteria for cocaine dependence has considerable advantages and may capture an intermediate step in the transition from controlled, occasional cocaine use to compulsive drug use characteristic for dependent cocaine use. Moreover, despite the relatively high prevalence rates indicating that there are large numbers of recreational cocaine users, merely no knowledge exists to date if even non-dependent, recreational cocaine users manifest molecular dopaminergic and glutamatergic adaptations as well as behavioural changes in the form of decision-making deficits and altered social interaction behaviour. Therefore, elucidating if deficits already occur with recreational cocaine use patterns can be of utmost importance to provide sound information for effective prevention strategies that may particularly sensitize young adolescents prone to drug exposure. A further advantage that should be emphasized is the fact that recreational cocaine users manifest fewer psychiatric co-morbidities, less pronounced co-use of other illicit substances, and exhibit a higher level of general functioning including better health, occupational and social integration in comparison to dependent cocaine users, reducing the number of confounding factors. Second, the present studies are unique due to objective hair toxicology analyses encompassing drug use over the past six months and allowing exclusion of participants who had used undesired substances such as MDMA and opiates or who had not used cocaine at all despite claiming so in the drug interview. Third, because psychiatric co-morbidities such as attention deficit hyperactivity disorder (ADHD) and depression frequently occur in cocaine users (Klassen et al., 2012; Perez de Los Cobos et al. 2011), symptoms of ADHD and depression were assessed and introduced into the statistical analyses. In addition, comprehensive psychiatric diagnostic procedures were carried out in order to exclude severe psychiatric diagnoses including schizophrenia and obsessive-compulsive disorder as well as a known family history of severe psychiatric disorders. Forth, post-acute drug effects as measured by positive urine toxicology as well as current craving urges were assessed and taken into account in statistical analyses. Finally, the sample sizes were relatively large for the addiction field, particularly in the third study where we assessed decision-making in 68 recreational cocaine users, 30 dependent cocaine users, and 68 drug-naïve control subjects, but also in the PET study comprising 18 cocaine users and 18 controls.

The most important limitation applying to all of the present studies is the cross-sectional design precluding a conclusive statement if neuroadaptations and behavioural alterations in cocaine users are in fact cocaine-induced or may rather be due to pre-existing vulnerabilities that may become exacerbated with drug use. A number of seminal studies have indeed suggested that drug users exhibit

pre-existing cerebral and behavioural alterations that may render individuals more prone to initiate drug use and to display behavioural deficits (Ersche et al., 2010, 2012*a*, *b*, *c*; Kreek et al., 2005). Contrariwise, preclinical studies and to some extent also human neuroimaging studies have provided evidence for long-lasting cocaine-induced neuroadaptations, entailing deleterious behavioural consequences (for reviews, Goldstein and Volkow, 2002, 2011; Kalivas, 2009; Kalivas and O'Brien, 2008; Koob and Volkow, 2009; Volkow et al., 2011). Moreover, cognitive and non-social decision-making deficits, as well as colour vision impairment observed in the first and third study of the present thesis correlated negatively with cumulative cocaine use and duration of use indicating that longer and more pronounced cocaine use may indeed have an effect on these measures. Alternatively, an interaction between pre-existing vulnerabilities and adverse drug-induced effects appears likely. However, to conclusively unravel the direction of causality, longitudinal and prospective studies are required.

A number of limitations apply to specific aspects of the three studies conducted in this thesis, of which the most pertinent ones shall be briefly designated. It is noteworthy that colour discrimination is not exclusively influenced by cocaine use, but may also be affected by age, alcohol and nicotine use (Djamgoz et al., 1997; Hart, 1987; Masson et al., 1993; Witkovsky, 2004). Despite carefully matching the drug user groups with the control group for the aforementioned variables and controlling potentially confounding variables in statistical analyses, we cannot fully exclude the possibility that these factors may have contributed to alterations in colour discrimination. Furthermore, although preclinical and clinical evidence supports the notion that colour discrimination may be partially mediated by dopaminergic neurotransmission in the retina, as we did not have a direct measure of retinal dopamine, we cannot exclude that other mechanisms may have played a role in impaired colour discrimination in cocaine and amphetamine users. One confounding source could have been the occurrence of minor ocular blood vessel bursts in stimulant users. In addition to alterations in retinal dopamine neurotransmission, sub-cortical and cortical processing may also have contributed to decreased colour discrimination ability (Conway et al., 2010).

A potential drawback of the PET study can be seen in the lacking arterial blood sampling and the reliance on using the cerebellum as reference region. However, although the human cerebellum may not be entirely devoid of mGluR5s (Patel et al., 2007), in vitro and in vivo studies have provided evidence for negligible mGluR5 binding in the cerebellum (Elmenhorst et al., 2010; Hamill et al., 2005) and hence that using the cerebellum as a reference region may be feasible to quantify mGluR5 density (Barret et al., 2010). A second drawback arises due to the limited spatial resolution inherent to PET imaging, wherefore it was impossible to specifically quantify mGluR5 availability in the NAcc core and shell regions as is possible in preclinical studies. Therefore, we cannot entirely rule out that cocaine users may have exhibited a subtle difference in mGluR5 availability in these NAcc sub-regions compared to controls. Furthermore, despite adequately matching cocaine users and controls

for duration and frequency of smoking, the strong influence of recent nicotine use on mGluR5 availability may have masked less pronounced effects associated with cocaine use. In addition, the cross-sectional design does not conclusively answer if the reduced mGluR5 density in smokers constitutes a pre-existing or nicotine-induced effect, however the correlation with nicotine abstinence duration suggests the latter to be more likely. Lastly, a number of studies have reported that alcohol use is associated with opposing effects on non-synaptic, extracellular glutamate levels, resulting in increased basal glutamate levels in the NAcc, hippocampus, amygdale, and dorsal striatum (Dahchour and De Witte, 2003; Melendez et al., 2005; Roberto et al., 2004; Rossetti et al., 1999). Finally, because cocaine users indicated to use more alcohol than controls, we cannot exclude the possibility that alcohol may have been a confounding factor. Though, we did not find correlations between mGluR5 availability and alcohol use or ethylcocaine.

With regard to the third study, we had to resort to using a cover story in the social interaction paradigms, which may have caused some of the participants to engage in different decision-making behaviour. However, after study completion, all participants were asked if they had doubts about interacting with a real person. Introducing this as a covariate did not change the results and interestingly, cocaine users reported even fewer doubts than controls about truly interacting with another person. In addition, the economic interaction tasks did not yield information about underlying reasons and motives why participants decided to distribute money in a fair or self-serving manner.

6.5 Implications for prevention and treatment strategies

6.5.1 Screening tools for dopaminergic alterations in cocaine users

One of the original ideas fostering the interest to examine colour discrimination in cocaine users was the notion that the detection of colour vision discrimination impairment may serve as a suitable indirect neurobiological marker for central dopamine function in cocaine-dependent patients. More specifically, Roy and colleagues (1997*a*, *b*, 2003) proposed that electroretinogram (ERG) blue cone b-wave amplitudes may be utile as a neurobiological central dopamine marker in cocaine-dependent patients. Equally interesting is the fact that ERG blue cone b-wave amplitudes below 0.5 mV were associated with stronger craving in dependent cocaine users, corresponding to reports that D_2 receptor binding in the dorsal striatum during a cocaine-cue condition was linked to subjectively experienced cocaine craving (Volkow et al. 2006). A screening tool enabling the detection of changes in retinal dopamine neurotransmission that may be linked to central dopamine alterations, associated cognitive deficits, and craving urges, would be of great interest due to substantially lower costs and due to being less intrusive compared to PET imaging. However, implications regarding the usefulness of the

Lanthony Desaturated Panel D-15 (LD-15) colour discrimination task applied in the first study of the present thesis are limited. Detailed analyses addressing its utility as a potential screening tool revealed satisfactory specificity, thus ample ability to distinguish between different substances (cocaine/amphetamine vs. MDMA/no drugs), whereas the sensitivity of the LD-15 is not sufficient for diagnostic and predictive purposes in stimulant users. On that account, the LD-15 used in the first study is not sensitive enough to serve as a screening tool for colour vision impairment and cognitive deficits related to stimulant use. The utility of the ERG blue cone amplitudes as a potential screening tool, perhaps in combination with other markers, remains to be determined.

6.5.2 Pharmacotherapies aimed at reinstating the glutamate homeostasis and plasticity

Despite the debilitating threat that cocaine addiction poses on society and economy, effective pharmacological treatment is still lacking. The advancing understanding with regard to enduring glutamatergic neuroadaptations in preclinical cocaine addiction models that may be responsible for the persisting nature of cocaine dependence and drug addiction in general, provides the unique prospect to develop pharmacotherapies aimed at facilitating lasting abstinence. The findings from the second study of the present thesis have important implications for the development of medications directed at facilitating smoking cessation. The global, very pronounced decrease of mGluR5 availability in smokers is likely to reflect a compensatory, protective mechanism inhibiting relapse as has been suggested for cocaine-related mGluR5 down-regulation observed in the rat NAcc (Kalivas, 2009). Upon repeated stimulation with nicotine, mGluR5s might undergo internalization and may subsequently be recycled to the cell surface after a certain abstinence period. mGluR5 antagonists might further strengthen the hypothesized protective effect of down-regulation. Although we did not find differences in mGluR5 availability associated with cocaine use, preclinical studies have revealed that blocking of mGluR5s was linked to attenuated self-administration and reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin seeking (Backstrom and Hyytia, 2006; Besheer et al., 2008; Gass et al., 2009; Paterson and Markou, 2005; Paterson et al. 2003; Platt et al., 2008; van der Kam et al. 2007). Accordingly, it seems likely that mGluR5 antagonists may be efficacious to prevent relapse in human drug users as well. Therefore, when pharmacological compounds enter clinical trials and become approved for application in humans, it should be systematically investigated if mGluR5 antagonists indeed facilitate abstinence in cocaine and nicotine users as well as users of other types of addictive drugs, and which users may benefit the most, perhaps also depending on their stage in the addiction cycle. Preclinical research has shown that negative allosteric modulators targeting mGluR5s inhibited drug administration and relapse, whereas positive allosteric modulators facilitated extinction, but also promoted relapse (Kalivas and Volkow 2011). Currently, no clinical studies with

drug-addicted individuals are being carried out, however, a number of ongoing clinical trials investigate negative allosteric modulators of mGluR5s in the context of other diseases (Berry-Kravis et al., 2009; Zerbib et al., 2010).

Apart from mGluR5s as a potential pharmacological target, several other mechanisms implicated in establishing the glutamate homeostasis as well as in compensatory mechanisms could be targeted with medications. For instance, in preclinical models, administration of N-acetylcysteine not only normalized its molecular target, the cystine-glutamate exchanger, but also restored several cocaine-induced adaptations involved in establishing glutamate homeostasis and in regulating synaptic plasticity (Moussawi et al., 2011). Moreover, first clinical trials applying N-acetylcysteine in humans addicted to cocaine and nicotine were successful in reducing the cue-induced desire to use cocaine, cocaine-induced craving, and the number of cigarettes smoked (Amen et al., 2011; Knackstedt et al., 2009; LaRowe et al., 2007). Targeting other receptor proteins has been associated with further benefit in preclinical studies. Accordingly, mGluR2/3 agonists (Gass and Olive, 2008), stimulators of GLT-1 (Knackstedt et al., 2010; Lewerenz et al., 2009; Sari et al., 2009), and AMPA antagonists may inhibit relapse (Backstrom and Hyytia, 2007; Di Ciano and Everitt, 2001; Sanchis-Segura et al., 2006), and partial NMDA agonists may facilitate extinction (Myers et al., 2011). Combinations of medications targeting different molecular mechanisms might be more clinically effective and permit administration of lower, better tolerable doses (Kalivas and Volkow, 2011).

6.5.3 Prevention and treatment strategies to tackle cocaine addiction

The results from the third study of the present thesis have important implications for the conceptualization of prevention and treatment approaches with the superordinate goal of augmenting quality of life and successful social integration. Vulnerability markers identified in decision-making paradigms in cocaine users could be specifically addressed and incorporated into treatment programs. Analogous to offers in schizophrenia treatment, the potential of cognitive remediation strategies has recently been emphasized for the addiction field. Accordingly, it was shown that working memory training significantly decreased the propensity to discount delayed rewards in stimulant addicts (Bickel et al., 2011). As evidenced by our results, even recreational cocaine users exhibit a relative inability to forgo smaller immediate rewards in favour of larger delayed rewards in the intertemporal discounting paradigm. This is likely to apply to everyday life situations as well in cocaine users in the form of being unable to refrain from taking cocaine in favour of later, more complex benefits including perpetuating a satisfactory family life, managing occupational demands, and maintaining physical and mental health, which are quintessential problems of cocaine addicts. Advocating awareness of this problem, acquiring new cognitive and behavioural alternatives, as well as establishing strategies how to endure states of intense craving may in combination with

pharmacotherapies contribute to developing and maintaining successful abstinence. Moreover, cognitive remediation approaches may help preventing recreational cocaine users from making the transition to addiction.

In addition, maladjustments in social cognition have been shown to adversely impact the development, course, and outcome of psychiatric conditions and may hamper addiction treatment substantially. In light of the high relevance of intact social cognitive abilities to adequately navigate through interactive everyday life situations and in the long run to maintain healthy relationships within family and the occupational setting, cocaine users may benefit from social skills training programs. Such programs could incorporate specific exercises aimed at improving social cognitive abilities including adopting another person's perspective during complex social situations, aiding to experience empathic reactions for social interaction partners, eventually leading to more adequate behavioural responses.

6.6 Unresolved issues and future directions

It was mentioned in the introduction of the present thesis that the empirical studies are part of a comprehensive longitudinal study. It is of utmost importance to address the causal direction of whether cognitive and decision-making deficits as well as molecular alterations are cocaine-induced or due to predisposition. Our longitudinal study may provide first insight into this yet unresolved issue. If cocaine users drastically increase their drug use (making the transition to dependent cocaine use according to DSM-IV criteria) over the investigated period of one year and then show significantly more pronounced deficits in the second assessment, or alternatively, if cocaine use is stopped or significantly decreased and the reduction may be associated with improved task performance in the second assessment, a first indication could be derived about causality and reversibility of deficits. However, even longitudinal studies cannot fully answer the question of causality. Therefore, prospective studies should be carried out in addition. Moreover, studies with twins, who generally share many similar environmental, social, and genetic factors and therefore experience more homogenous conditions, could further broaden the knowledge about vulnerability and protective factors that may explain why one twin engages in drug use, while the other does not. Overall, beyond the question of causality, longitudinal, prospective, and twin studies may further allow the identification of vulnerability markers predicting the initiation of drug use and the transition to dependence.

Furthermore, if the necessary knowhow and budget to perform hair toxicology analyses is available, all future studies in the addiction field should objectively identify the drugs participants used over the past six months. Hair toxicology analyses improved the validity of our study

substantially and provided the unique prospect to control for poly-toxic drug use, which constitutes one of the major confounding factors in addiction research and makes it difficult to appraise the risk for detrimental physical and psychological health effects posed by one particular class of illegal substances. In fact, 20% of the participants in our study had to be excluded, either due to poly-toxic drug use or lack of cocaine and its metabolite in the hair samples. In the specific case for cocaine, future studies could additionally analyze if hair samples contain traces of dangerous diluents and adulterants such as levamisol (Wolford et al., 2012).

The findings of the first study evidently point out that a more complete understanding should be strived for regarding the mechanism of how dopamine is involved in colour discrimination processing in general and why it is relatively specific for blue-yellow colour vision as well as how cocaine may precisely impact retinal neurotransmission. In order to gain insight into these molecular mechanisms, more preclinical research is needed. In addition, further knowledge should be acquired to what extent retinal and cerebral dopamine may be related and how closely they are associated with cognitive functions. One possibility to address this issue would be to conduct a PET study where D_2/D_3 receptor availability in the striatum could be related to ERG blue cone b-wave amplitudes (scores in the LD-15 and further ophthalmological measures) and cognitive measures in cocaine users and drug-naïve controls, perhaps in combination with a pharmacological challenge. Additional information could be derived from post-mortem studies where retinal dopamine levels in cocaine users could be compared to retinal dopamine levels in controls. Future research efforts should be devoted to combining clinical tests with more complex psychophysical measures in order to understand the underlying mechanisms better by which alterations in dopamine transmission may affect colour vision processing.

Findings regarding cocaine-induced alterations in the glutamate system have almost exclusively been derived from preclinical studies. Accordingly, very little is known to what extent these molecular adaptations may extend to human cocaine users. Therefore, future research should attempt to gain more insight into potential alterations in the glutamate circuitry in human cocaine users and drug users in general. Due to ethical barriers impeding direct experimental manipulation in humans, multimodal imaging may be the most sophisticated way of achieving this goal. For instance, it could be informative to investigate how free glutamate levels in the NAcc, ACC, and PFC, measured by means of MRS, may be related to mGluR5 availability, PFC metabolism, and resting state activity in different stages of addiction (acute drug intake, withdrawal, craving). Moreover, as stated in section 6.5.2, the results from the second study do not answer if pharmacological compounds targeting the mGluR5 in cocaine users and smokers have the ability to successfully prevent relapse. In general, once medications that target glutamatergic mechanisms become clinically available, future studies should not only test their efficacy for treating cocaine and nicotine addiction by means of assessing successful abstinence, but could also attempt to measure the associated molecular alterations brought upon by the pharmacological intervention. For this purpose, apart from the aforementioned

multimodal imaging, studies investigating molecular alterations before and after pharmacological intervention are likely to be a promising approach. Accordingly, the effect of N-acetylcysteine on free glutamate levels in the NAcc in cocaine users could directly be compared to free glutamate levels in the same subjects before the treatment with N-acetylcysteine. In addition, it would be possible to test if cue-induced cocaine craving is indeed associated with increased free glutamate levels in the NAcc in dependent cocaine users and if medicated cocaine users may exhibit a less pronounced glutamate increase. Lastly, our results from the PET study are consistent with a recent preliminary study providing evidence that nicotine use has a marked impact on mGluR5 availability (Akkus et al., 2013). Preclinical findings have shown that some molecular mechanisms in the glutamate circuitry become altered in a similar manner through the administration of nicotine and cocaine (e.g., down-regulation of xc^{-}). Hence, the question arises if co-use of different addictive substances may lead to more pronounced neuroadaptations, and in turn to more severe addiction, strongly impeding abstinence and also if a combination of pharmacological therapies would be advisable to alleviate stronger combined adverse effects.

With regard to social decision-making in cocaine users, future research should strive to understand if already more basal mechanisms of social cognitive abilities including emotion perception, theory-of-mind, empathy, which are a prerequisite of social interaction, are affected. In fact, data from our lab suggest that recreational and dependent cocaine users exhibit less emotional empathy in comparison to controls, whereas the more basal emotion recognition does not seem to be compromised. Interestingly, dependent cocaine users over-interpreted situations where theory-of-mind was necessary (Preller et al., submitted). Moreover, it is essential to gather more information about underlying motives and reasons why cocaine users decide to behave in a more self-serving manner in economic money distribution tasks. A myriad of reasons could apply ranging from merely needing more money to buy the drug to satisfy craving urges to the inability of adopting the other person's perspective or lack of empathy. The fact that cocaine users feature a 22-fold increased risk for an antisocial personality disorder (Rounsaville, 2004), which is consistent with higher scores in scales measuring antisocial behaviour in cocaine users of our study, and the fact that social decision-making did not correlate with drug use patterns in our study may indicate that pre-existing personality traits could be responsible for the observed more self-serving behaviour. These results further imply the necessity for longitudinal and prospective studies assessing the question of causality. Advances in the field of neuroeconomics have stressed the importance of measuring decision-making behaviour in combination with analyzing neuronal activity. Accordingly, fMRI during decision-making could further contribute to a more complete understanding how drug users differ in cerebral activity patterns compared to controls. Another source of information that has not sufficiently been taken into account in addiction research is reliance on social surroundings including families, friends, and therapists. These social groups could provide valuable information about specific areas and situations where

cocaine users may encounter everyday life difficulties and exhibit social deficits as well as if these maladjustments are assumingly due to pre-existing deficits, acute or long-term drug effects, craving, or anhedonia in withdrawal phases. Moreover, evaluating the composition of the social environment itself could provide valuable information if cocaine users have smaller social networks, how much support they receive, and how well they are integrated in occupational and social structures as well as how the social network could help addicted individuals to successfully achieve and maintain abstinence.

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Vonmoos M, **Hulka LM**, Preller KH, Minder F, Baumgartner MR, Quednow BB (2013). Cognitive Impairment in Cocaine Users is Drug-Induced but Partially Reversible: Evidence From a Longitudinal Study. *(submitted).*

CONFERENCE PRESENTATIONS

Talks

Hulka LM, Eisenegger C, Preller KH, Vonmoos M, Jenni D, Quednow BB. *Social and non-social decision-making deficits in recreational and dependent cocaine users.* ISAM, Geneva, Switzerland (16.10.2012).

Hulka LM, Wagner M, Preller KH, Jenni D, Quednow BB. *Blue-yellow colour vision impairment and cognitive deficits in occasional and dependent stimulant users.* Leiden University, Netherlands. (25.1.2012; *invited talk*)

Hulka LM, Eisenegger C, Preller K, Bendrick K, Jenni D, Quednow BB. *Social and non-social decision-making in occasional and dependent cocaine users.* DGPPN, Berlin, Germany (25.11.2011).

Hulka LM, Roder V, Müller DR. *Effektivität der Integrierten Neurokognitiven Therapie (INT): Ergebnisse einer Multizenterstudie in der Schweiz, Deutschland und Österreich.* DGPPN, Berlin (26.-29.11.2008).

Posters

Hulka LM, Treyer V, Scheidegger M, Johayem A, Preller KH, Vonmoos M, Ametamey SM, Buck A, Seifritz E, Quednow BB. Nicotine but not cocaine use is associated with decreased metabotropic glutamate receptor 5 density in humans. SOBP, Annual Meeting, San Francisco, USA (16.5.-18.5.2013).

Hulka LM, Scheidegger M, Henning A, Preller KH, Vonmoos M, Ametamey S, Buck A, Seifritz E, Quednow BB. *Metabotropic glutamate receptor 5 densities and free glutamate concentrations in occasional and dependent cocaine users.* CINP, 28th World Congress, Stockholm, Sweden (3.6.-7.6.2012).

Hulka LM, Wagner M, Preller K, Jenni D, Kühn KU, Maier W, Quednow BB. *Blue-yellow colour vision impairment in occasional and dependent stimulant users.* ZNZ Symposium, Zurich, Switzerland (16.9.2011).

Hulka LM, Eisenegger C, Preller K, Bendrick K, Jenni D, Quednow BB. *Social and non-social decision-making in occasional and dependent cocaine users.* 24th ECNP, Paris, France (5.9.2011).

Hulka LM, Eisenegger C, Preller K, Jenni D, Quednow BB. *Soziales und nicht-soziales Entscheidungsverhalten bei nicht-abhängigen und abhängigen Kokainkonsumenten.* 37. Tagung Psychologie und Gehirn, Heidelberg, Germany (23.6.2011).

Hulka LM, Eisenegger C, Preller K, Seifritz E, Quednow BB. *Social decision-making and impulsivity in recreational and dependent cocaine users.* Tag der Forschung, University Hospital of Psychiatry, Zurich, Switzerland (2.12.2010).

Hulka LM, Wagner M, Kühn KU, Maier W, Quednow BB. *Impaired colour vision in recreational stimulant users.* 23th ECNP Congress, Amsterdam, Netherlands (28.8.-1.9.2010).

Hulka LM, Eisenegger C, Preller K, Jenni D, Seifritz E, Quednow BB. *Social decision-making and impulsivity in recreational and dependent cocaine users.* ZNZ Symposium, Zurich, Switzerland (17.9.2010).

Hulka LM, Wagner M, Kühn KU, Maier W, Quednow BB. *Der Konsum von Stimulanzien beeinträchtigt das Blau-Gelb-Farbsehen.* 36. Tagung Psychologie und Gehirn, Greifswald, Germany (10.6.-12.6.2010).

Hulka LM, Wagner M, Kühn KU, Maier W, Quednow BB. *Impaired colour vision in cocaine and ecstasy users.* 30th SSBP Annual Meeting, Zurich, Switzerland (Nature and Nurture) (18.3.2010).

RESEARCH AWARDS

Lundbeck Psychiatry Prize 2013

Zürich, 29.7.2013